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CONTENTS

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas.
From Proceedings of the American Academy of Arts and Sciences, Vol. 48, No. 18, pp. 669–678. April, 1913.

26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin: a Distinct Species.
From Journal of Morphology, Vol. 23, No. 4, pp. 625–636. December, 1912.

27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer.
From Proceedings of the American Academy of Arts and Sciences, Vol. 48, No. 20, pp. 737–773, 4 pls. May, 1913.

28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus.
From Proceedings of the American Academy of Arts and Sciences, Vol. 49, No. 10, pp. 569–626, 8 pls. October, 1913.

29. Hilton, W. A. — The Central Nervous System of Tunica nigra.
Zoologische Jahrbücher, Abteilung für Anatomie, Band 37, No. 1, pp. 113–130. December, 1913.

30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin.
From The Journal of Physiology, Vol. 47, No. 6, pp. 491–492. February, 1914.

31. Parker, G. H. — The Locomotion of Chiton.
2 p. April, 1914.

32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges.
From The Journal of Experimental Zoölogy, Vol. 16, No. 3, pp. 443–445. April, 1914.

33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig.
From Zoologische Jahrbücher, Abteilung für allgemeine Zoologie und Physiologie, Band 35, No. 3, pp. 233–297. August, 1915. *Reprinted September, 1917.*

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34. Crozier, W. J. — The Orientation of a Holothurian by Light.
    From The American Journal of Physiology, Vol. 36, No. 1, pp. 8-20. December, 1914.

35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda.
    From The American Naturalist, Vol. 49, No. 577, pp. 28-36. January, 1915.

36. Arey, L. B. — The Orientation of Amphioxus during Locomotion.
    From The Journal of Experimental Zoölogy, Vol. 19, No. 1, pp. 37-44. July, 1915.

37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw.
    From The American Journal of Physiology, Vol. 36, No. 2, ff. 196-202. January, 1915.

38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana.
    From Proceedings of the American Academy of Arts and Sciences, Vol. 51, No. 9, pp. 183-211, 6 pls. December, 1915.

39. Crozier, W. J. — On Cell Penetration by Acids.
    From Science, New Series, Vol. 42, No. 1090, pp. 735-736. November 19, 1915.

40. Crozier, W. J. — Regarding the Existence of the ' Common Chemical Sense ' in Vertebrates.
    From The Journal of Comparative Neurology, Vol. 26, No. 1, pp. 1-8. February, 1916.

41. Crozier, W. J. — Cell Penetration by Acids.
    From The Journal of Biological Chemistry, Vol. 24, No. 3, pp. 255-279. March, 1916.

42. Hecht, S. — The Water Current produced by Ascidia atra Lesueur.
    From The Journal of Experimental Zoölogy, Vol. 20, No. 3, pp. 429-434. April, 1916.

43. Crozier, W. J. — The Rhythmic Pulsation of the Cloaca of Holothurians.
    From The Journal of Experimental Zoölogy, Vol. 20, No. 3, pp. 297-356. April, 1916.

44. Crozier, W. J. — Some Indicators from Animal Tissues.
    From The Journal of Biological Chemistry, Vol. 24, No. 4, pp. 443-445. April, 1916.

45. **Galloway, T. W., and Welch, P. S.** — Studies on a Phosphorescent Bermudian Annelid, Odontosyllis enopla Verrill.

From Transactions of the American Microscopical Society, Vol. 30, No. 1, pp. 13–39, 5 pls. January, 1911. *Reprinted June, 1916,* 26 pp., 5 pls.

46. **Crozier, W. J.** — The Taste of Acids.

From The Journal of Comparative Neurology, Vol. 26, No. 4, pp. 453–462. August, 1916.

47. **Wenrich, D. H.** — Notes on the Reactions of Bivalve Mollusks to Changes in Light Intensity: Image Formation in Pecten.

From The Journal of Animal Behavior, Vol. 6, No. 4, pp. 297–318, July–August, 1916.

48. **Crozier, W. J.** — Cell Penetration by Acids. II. Further Observations on the Blue Pigment of Chromodoris zebra.

From The Journal of Biological Chemistry, Vol. 26, No. 1, pp. 217–223. September, 1916.

49. **Crozier, W. J.** — Cell Penetration by Acids. III. Data on some Additional Acids.

From The Journal of Biological Chemistry, Vol. 26, No. 1, pp. 225–230. September, 1916.

50. **Crozier, W. J.** — On a Barnacle, Conchoderma virgatum, attached to a Fish, Diodon hystrix.

From The American Naturalist, Vol. 50, No. 598, pp. 636–639. October, 1916.

51. **Crozier, W. J.** — On the Immunity Coloration of some Nudibranchs.

From Proceedings of the National Academy of Sciences, Vol. 2, No. 12, pp. 672–675. December, 1916.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
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No. 25.

PRELIMINARY STUDY OF THE SALINITY OF SEA-WATER IN THE BERMUDAS.

By Kenneth L. Mark.

With Three Maps.

From the Proceedings of the American Academy of Arts and Sciences,
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PRELIMINARY STUDY OF THE SALINITY OF SEA-WATER IN THE BERMUDAS.[1]

By Kenneth L. Mark.

Presented by E. L. Mark, January 8, 1913. Received February 3, 1913.

The objects of this investigation of the salinity and of the temperature of the waters in and about the Bermudas were the collection of data which would supplement those recorded for other parts of the Atlantic Ocean, especially by the "Conseil Permanent International pour L'Exploration de la Mer," and the study of the relation of the salinity to the depth below the surface, to the depth of the sea, and to the locality. A knowledge of these relations was desired as a part of the basis for studies on the distribution of oceanic organisms at the Bermudas.

For these purposes, therefore, samples of water were collected at various places and depths and the temperature of the water was noted in each case. The salinity of these samples was determined by the method used by the "Conseil International." This consists of the complete precipitation of the halides of the sea-water by the requisite amount of a standard solution of silver nitrate. The salinity and density of the samples are then calculated from the analytical results by the aid of the Hydrographical Tables of Knudsen.

Procedure.

The water was collected in a Buchanan[2]-Nansen[3] stop-cock water-bottle, as modified by Dr. H. B. Bigelow,[4] which allows the free passage of water through it during its descent, but can be made to enclose a sample of water at any desired depth. The water was immediately transferred through a brass cock to glass bottles. Care was taken to allow as little evaporation as possible during this transfer. The glass bottles were provided with porcelain stoppers with rubber rings, held on by wire, like the old-fashioned beer-bottle stoppers. They were the so called "citrate of magnesia" bottles made by the Whitall, Tatum Co. The water was often stored in these bottles for several days before analyzing it.

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[1] Contributions from the Bermuda Biological Station for Research. No. 25.

[2] Challenger Report, Narrative, Vol. I, Part 1, p. 112–117.

[3] The Norwegian Sea, its Physical Oceanography based upon the Norwegian Researches 1900–1904, by B. Helland-Hansen and F. Nansen. Christiania 1909, *in* Report on Norwegian Fishery and Marine-Investigations, Vol. II, 1909, No. 2, p. 55.

[4] Dr. Bigelow's modification consists chiefly in the substitution of a messenger for the propeller used by Nansen, and will be described in a forthcoming report to be published in the Bull. Mus. Comp. Zoöl., Cambridge Mass.

The temperature of the water was determined by a Negretti and Zambra deep-sea thermometer, which was attached to the cable carrying the water bottle and directly beneath it. This thermometer had previously been compared with a thermometer standardized by the Deutsche Physikalische Technische Reichsanstalt.

The volumes of sea-water taken for analysis and the volumes of silver nitrate solution required to react with them were measured in a Knudsen pipette and a Knudsen burette respectively; both were made by R. Goetze, Leipzig. The former is an ordinary pipette of about 25 c. c. capacity, provided with a three-way cock at the top. This arrangement allows the liquid to pass beyond the cock when the pipette is being filled; but upon turning the cock so that the body of the pipette is in connection with the air through its third opening, the pipette empties itself and the excess of liquid remains behind. Thus an exact filling is always attained. The Knudsen burette also has a three-way cock at the top, which is used in the same way. It is filled through a side tube entering at the bottom. The lower part is graduated in terms of the standard used in Knudsen's Tables.[5] The volume between the smallest graduation marks is about .05 c. c. and the total capacity of the burette is about 42 c. c. The burette used in this investigation was carefully standardized and the graduations were found to be equal within the limit of accuracy of the readings.

A silver nitrate solution, containing about 42 grams of the salt per liter, was prepared and stored in a large bottle of brown glass. This bottle, which was placed on a shelf several feet above the table, was provided with a two-hole stopper, through one hole of which a glass tubule extended from the bottom of the bottle to the inlet tube at the bottom of the Knudsen burette. The other hole of the stopper was kept closed except during the filling of the bottle.

The solution was standardized as follows. A tube of standard sea-water, obtained from the "Conseil International" at Copenhagen, was opened and the Knudsen pipette was immediately filled from it. The water was run from the pipette into a beaker, allowing one minute for drainage, and three drops of a one percent sodium chromate solution were added as indicator. Silver nitrate solution was then run in from the burette, at first rapidly, but at the end drop by drop, until a faint reddish tinge in the precipitate was permanent for thirty seconds. This was taken as the end point. The difference between the volume of silver nitrate used, as expressed in burette divisions, and the figure accompanying the standard sample was the value "*a*" of the Knudsen tables. Obviously this method of standardization shows only the strength of the solution as compared with the standard upon which the Knudsen tables are based; but since the analyses also are expressed in terms of this standard, no further knowledge of the concentration is required.

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[5] Knudsen, Martin: Hydrographical Tables etc. Copenhagen, G. E. C. Gad, and London, Williams & Norgate. 1901. v + 63 pp.

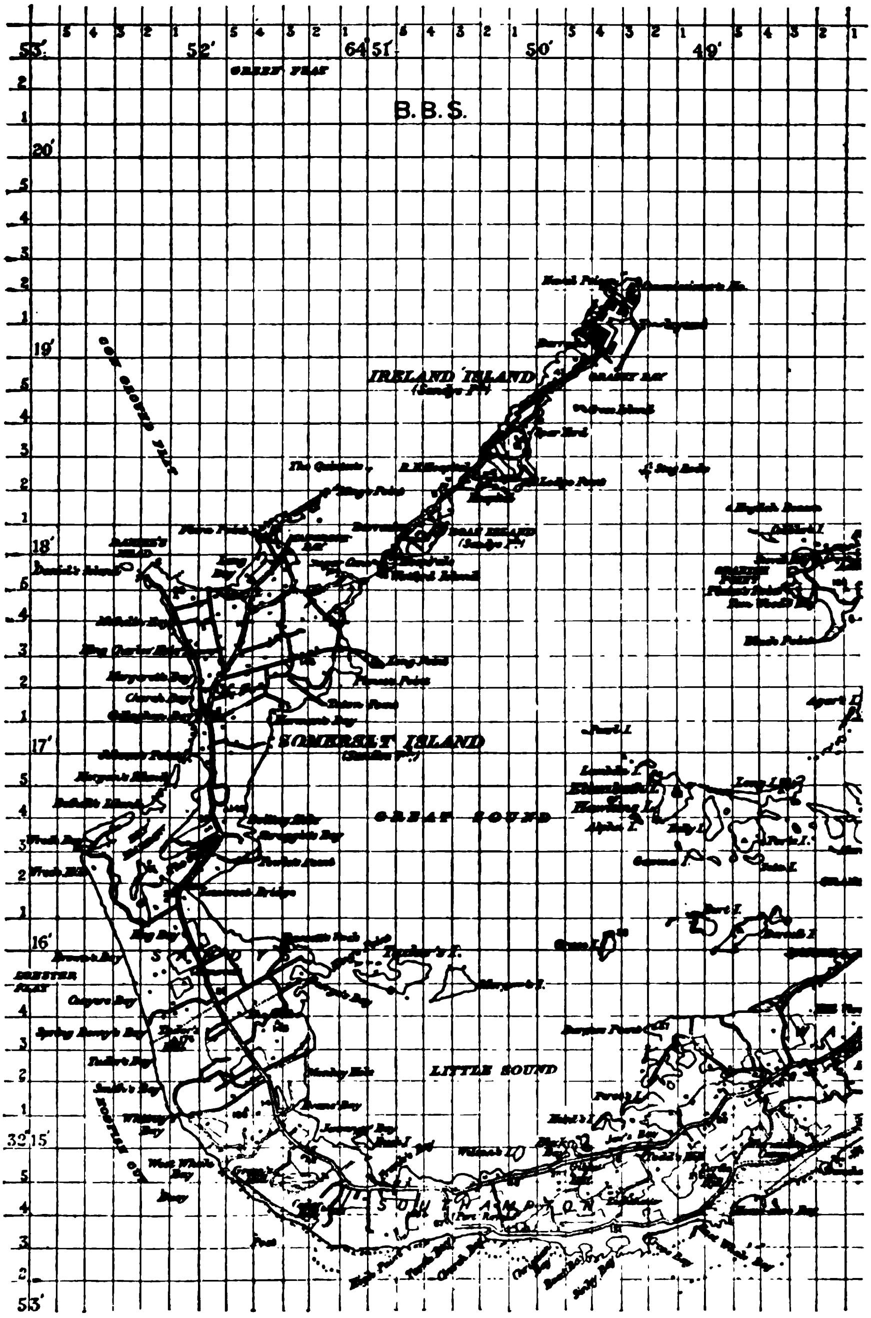
A secondary standard sea-water was prepared by diluting ordinary sea-water till approximately the same volume of silver nitrate was required to react with it as was required to react with the original standard. The exact ratio of these standards was determined with great care, since the secondary standard was the one constantly used during the investigation. At the end of the work, however, the silver nitrate was again compared with the original standard and was found to be unchanged.

In making a series of analyses, the silver nitrate solution was each day titrated against the standard water, as just described. The various samples of water were then titrated in an exactly similar way, and finally the solution was again compared with the standard. The temperature of the room was noted during the progress of the work, but in no case did it vary enough to require any correction of the results. All determinations were made in duplicate.

Accuracy.

Since the method of analysis consists of comparing the amount of silver nitrate solution necessary to react with a definite amount of sea-water with that necessary to react with the same amount of sea-water of known composition, no standardization of the pipette used for measuring the water was necessary. The amount delivered by the pipette was constant, as the time allowed for drainage was always the same.

The determination of the capacity of the burette in absolute units was not required. Only the relation of the divisions to each other had to be known, and these were found to be equal within the limit of accuracy to which the volume could be read. These readings could be relied upon to one one-hundredth of a unit. As the total volume of solution used in a determination was about twenty units, the



Map of S. W. third of the Bermuda Islands.

N. B. The latitude and longtitude of this and following maps is that of the British Ordinance Survey published in 1902.
The latitude of all places is about 18 seconds of arc less on the chart of the Hydrographic Office.

proportional error was thus one in two thousand. This measurement limited the reliability of the whole analysis, which was thus trustworthy to five hundredths of one percent.

Corrections for change of temperature are unnecessary when the standardization and analyses are carried out under conditions sufficiently similar. As the limit of accuracy of reading the burette was one in two thousand, this allowed a variation in temperature of 8° C., which was a greater change than ever took place.

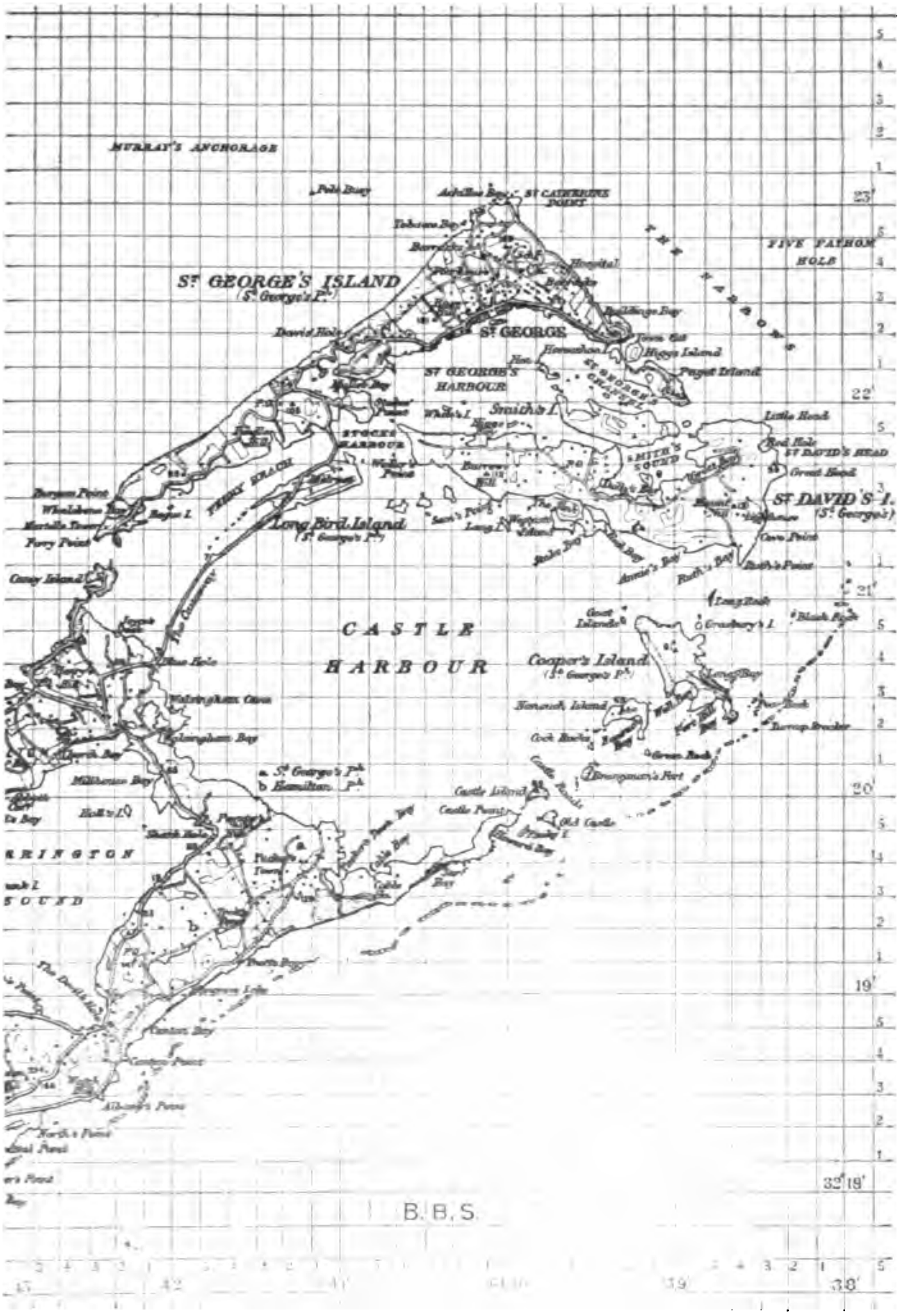
That no other sources of incidental error existed was shown by the facts that duplicate analyses always agreed to one part in two thousand or better, and that comparisons between the silver nitrate solution and the standard water always showed the same ratio to exist.

Table of Results.

Water Sample No.	Date	Locality			Depth		Temp. F.	Salinity
		Name	Latitude N.	Longitude W.	below surface	of bottom		
	July		32°	64°	fm.	fm.		
2	12	Brackish Pond Flats	21'15"	47'20"	1	7	82	36.47
3	12	Between No. 2 & Cobbler's Cut	19'45"	48'00"	7	8	81	36.58
4	13	Great Sound Sta. 1	16'10"	49'40"	6	7	82	36.83
5	16	" " " 2	17'10"	51'00"	8½	9	82	36.83
6	16	" " " 2			1		83	36.83
7	16	" " " 3	17'10"	50'20"	3½	4½	83.5	36.83
8	16	" " " 3			Surface		84	36.83
9	17	Little " " 2	15'40"	51'15"	5	6	84	36.87
10	17	" " " 1	15'05"	50'05"	Surface	6		34.92
11	19	Off Nonsuch Id. Sta. 0	19'46"	38'30"	10	ca. 12	81	36.51
12	19	" " " " 0			Surface		84	36.42
13	19	" " " " 1	19'44"	38'10"	18	ca. 20	80.5	36.45
14	20	Castle Harbor Sta. 0	19'50"	40'20"	6		83	36.49
15	20	" " " 1	20'05"	41'13"	6		83	36.51
16	20	" " " 1			Surface		84	36.51
17	20	Off Nonsuch Id. Sta. 2	19'36"	37'30"	50	ca. 275*	67	36.42
18	21	" " " " 1	19'44"	38'10"	20	ca. 30	81.5	36.45
19	22	" " " " 3	18'00"	36'10"	100	ca. 650*	64.5	36.42
20	28	Harrington Sd. Sta. 1	19'14"	42'56"	Surface	13	82	35.07
21	28	" " " 1			10		82	36.51
22	28	" " " 2	19'27"	42'30"	Surface	10½	82	35.52
23	28	" " " 2			9½		81	36.45
	Aug.							
24	2	" " " 1	19'14"	42"56"	Surface	12½	?	36.27
25	2	" " " 1			11½		83	36.45

The positions were usually determined by sighting conspicuous objects on shore.

The "depth below surface" and the "depth of bottom" were measured directly on the iron cable which carried the bottle. For the positions marked with an asterisk the depth of the bottom was not determined, but is that marked on the chart of the "Bermuda Islands"— issued by the Hydrographic Office, Washington, D. C., and corrected



Map of N. E. third of Bermuda Islands.

to 1900 — for the position indicated. The temperature and salinity were determined as described in the preceding pages.

Samples numbers 10, 20 and 22 were taken after heavy rains and therefore do not indicate the normal condition of the water. Samples numbers 24 and 25 were collected by E. L. Mark and were brought to Cambridge, where they were analyzed.

The pipette and burette used in Cambridge were not the ones used in Bermuda. The silver nitrate solution also was different and it was standardized against a different sample of Danish water. The agreement in the analytical results of samples 21 and 25, which were thus determined absolutely independently, serves to increase confidence in the reliability of all the analyses.

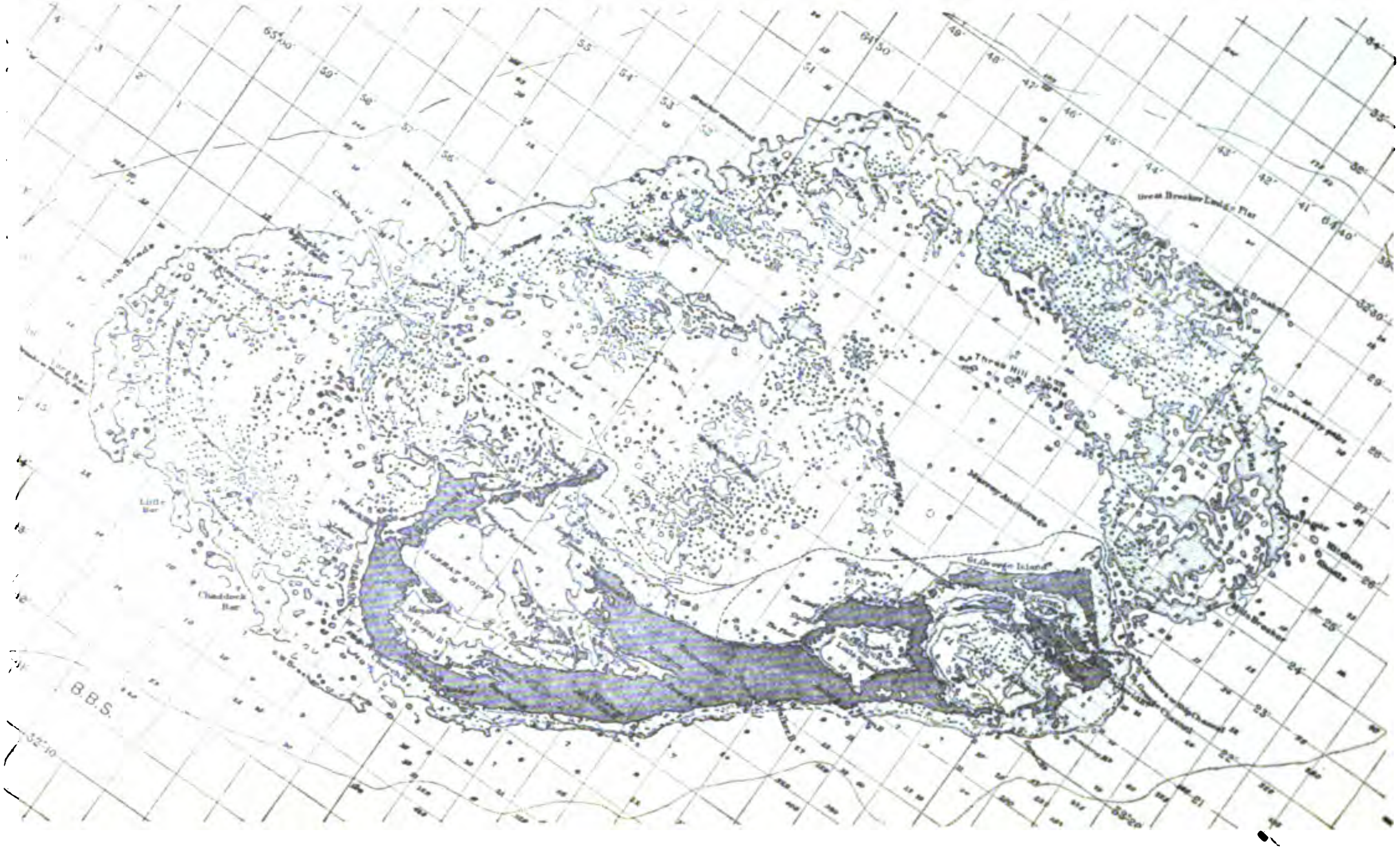
Discussion of Results.

The salinity of the water of the open ocean in the vicinity of Bermuda is undoubtedly that of the samples obtained off Nonsuch Island, namely 36.43 grams of salt per 1000 grams of sea-water. These samples were all collected outside the reefs, in positions exposed to the unbroken swell of the ocean from the south. In taking an average of the results, however, No. 19 has been omitted, as that sample was collected under unfavorable conditions. The depth below the surface, even down to 100 fathoms, appears to make no difference in salinity, except after recent rainfall.

The water of the shallow enclosed bays was found to increase in salinity with remoteness from the open ocean. This becomes particularly noticeable by comparing samples 2, 3, 4 and 9, where the successive samples were collected farther and farther within the shelter of the reefs and islands. The samples taken in Castle Harbor, also, were in good agreement with predictions based upon the connection of that bay with the ocean. The salinity of the water from the bottom of Harrington Sound, on the contrary, was surprisingly small, as compared with that of other enclosed bodies of water. It was found to be nearly the same as that of the open ocean, although the inlets to this sound are so narrow that the tide rises only about one fourth as much as it does outside.

Summary.

Data concerning the salinity and temperature of sea-water in the Bermudas are presented. These indicate that the salinity is independent of depth even down to 100 fathoms, but increases considerably as the water becomes more and more enclosed.



Map of the Bermuda Islands, reduced from the map of the Ordinance Survey.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., 31(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., 56(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., 38(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., 31(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., 40(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., 54: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., 46(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., 41(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., 42(18): 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., 42(19): 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., 33(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., 43(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, 13 (2): 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., 44(5): 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., 44(23): 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., 7(2): 189–238. 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, 1910 (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., 36 (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., 46(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., 47(7): 217–226, 4 pl. July, 1911.
22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. 10: 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, 29(1): 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., 47(16): 607–630. March, 1912.
25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., 48 (18): 669–678. April, 1913.

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9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. **Linton**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. **Parker**, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts and Sci., **43**(16) : 413–455. Apr., 1908.
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14. **Morgulis**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
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16. **Rand**, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2) : 189–238. 2 pls. Sept., 1909.
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    Separate : 6 pp. Nov., 1909.
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25. **Mark**, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
FOR RESEARCH.

No. 26.

CHROMODORIS ZEBRA HEILPRIN: A DISTINCT SPECIES.

By W. M. Smallwood and Elizabeth G. Clark.

From the Journal of Morphology, Vol. XXIII, No. 4.

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CHROMODORIS ZEBRA HEILPRIN: A DISTINCT SPECIES

W. M. SMALLWOOD AND ELIZABETH G. CLARK

Contributions from the Bermuda Biological Station for Research No. 26, and from the Zoological Laboratory of Syracuse University

SIX FIGURES

The following paper supplements one published by the senior author (Smallwood, '10). The two papers present the general morphology of Chromodoris zebra and point out the characters which distinguish it from C. villafranca. Bergh ('92) believed the two to be identical. The first paper gave a description of the external anatomy of C. zebra—the present one, the internal anatomy of the species. While our study of the internal anatomy has shown no marked deviation from the general plan of organization of the Doridae,—so well described by Eliot ('10, pp. 36 to 49) with Doris tuberculata as example,—it has furnished information concerning the anatomical details of this species not hitherto published.

DIGESTIVE SYSTEM

The mouth leads into a short but rather wide atrial chamber (6 mm. long by 9 mm. in diameter), the inner wall of which is lamellated. At the posterior end of this chamber a ring-shaped depression forms the boundary between the lateral wall and the posterior wall, the latter constituting the labial disc. A vertical slit-like opening in this disc is the entrance to a passage extending posteriorly for 4 mm.; this passage is lined with closely set spines, which form what is sometimes incorrectly termed a 'prehensile collar.' This spinous passage expands at its posterior end into the main buccal cavity, 5 mm. in length by 6 mm. in diameter. The floor of the main buccal cavity is formed by the odontophore, a round muscular prominence bearing the radula and divided into lateral halves by a longitudinal furrow. The walls of the buccal cavity are chiefly composed of muscles and are greatly thickened ventrally. The entire mass is cylindrical (fig. 2, *phx.*).

The radula, borne upon the rounded prominence forming the floor of the buccal cavity, is folded in the middle and fits into the longitudinal groove; it emerges from the curved sac which lies at the ventro-posterior angle of the buccal mass. This sac is lined with cells whose special function is the secretion of new teeth. The total number of rows of teeth is 95 to 100; of these 20 to 30 are in the radula sac, the posterior 4 to 5 being in process of formation. The median tooth is absent. In each transverse row are 165 to 170 teeth on each side. All of the pleural teeth bear two denticles; one long denticle terminating in a sharp slender point, and a shorter one with a slightly blunter distal end (fig. 3). Slight variations in size and form of teeth occur, as may be seen in figure 3, and as they near the margin, we find that the denticles gradually become more and more blunt, until at the outermost edge, they are mere rounded knobs supported by the broader base. In some teeth, the denticles are entirely lacking, doubtless having been worn off. The posterior margin of the tooth, that is, the side toward which the denticles point, is slightly crenulated. All teeth are of nearly the same size, measuring 20 micra long, 9 micra wide, and 3 micra thick. The longest denticle measured was 6 micra long.

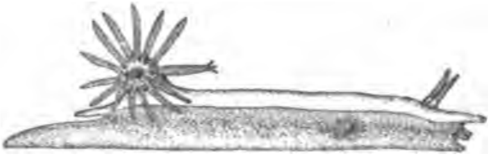
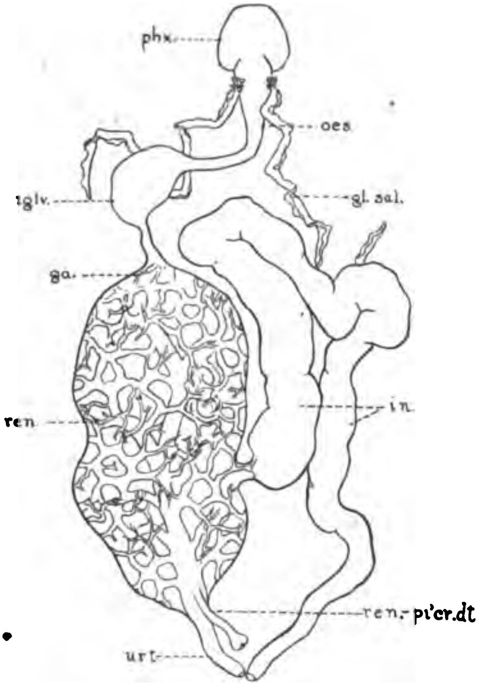


Fig. 1 Chromodoris zebra Heilprin. Viewed from the right side; the branchial-rosette turned toward the observer; $\frac{3}{2}$ natural size. Copied from Smallwood, '10.

Bergh ('78, pp. 23, 29 and 30) gives the following for Chromodoris villafranca:

> Forma corporis gracilis. Pedamentum foliorum branchialium humile. Color paginae superioris glaucescens, fasciis transversalibus latioribus caeruleis dilutis et praesertim lineis fulvis (ut plurimum 7) non semper



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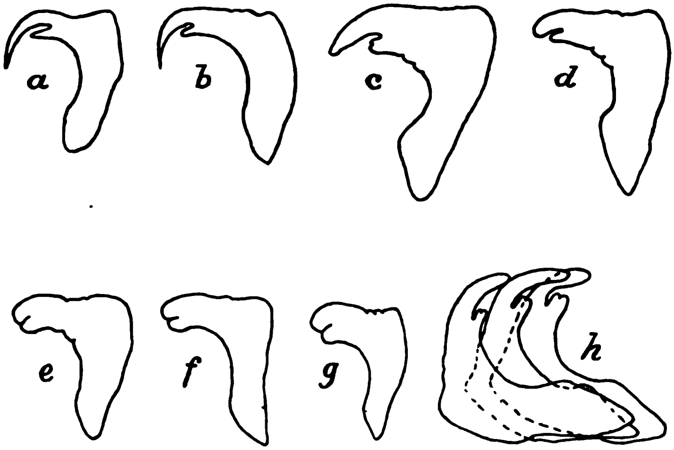

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Fig. 2 Digestive and renal systems with the intestine displaced; *ga.*, stomach; *gl.sal.*, salivary gland; *iglv.*, crop; *in.*, intestine; *oes.*, oesophagus; *phx.*, buccal mass; *ren.*, kidney; *ren.-pi'cr.dt.*, reno-pericardial duct; *urt.*, ureter.

Fig. 3 Pleural teeth, showing the successive steps in loss of denticles from *a* to *g*, also the arrangement of the teeth in relation to each other.

inter se distinctis et saepe divisis ornatus; margo dorsalis fulvus; clavus rhinophoriorum caeruleus, margine posteriore linea fulva ornatus; folia branchialia rhachide extus linea punctorum fulvorum ornata; latera corporis caerulescentia lineis fulvis 3–4 longitudinalibus (ut in dorso) ornata; podarium infra caerulescens vel glaucescens.

Dentes radulae grosse denticulati.

Hab. M. mediterr. (M. adriat., Napoli, Panormi, Genova, Bona).

Die Mundröhre etwa 4 mm. lang, von schön grün-blauer Farbe, ganz wie bei der vorigen Art [C. elegans]. Der Schlundkopf etwa 6 mm. lang bei einer Breite von 3 und einer Höhe bis 3 mm.; die Raspelscheide hinten und unten am Schlundkopfe etwa 1 mm. hervortretend, mit der schmutzig gras-grünen Raspel stark hindurchschimmernd. Die Lippenplatten grau-gelb, sonst ganz wie bei der vorigen Art; die Elemente derselben ganz wie bei jener, vielleicht etwas deutlicher am Grunde des Hakens gestreift. Die Zunge wie oben; die Raspel zeigte 26 Zahnplattenreihen, von denen die ersten 5 incomplet; weiter gegen hinten fanden sich noch 32 entwickelte und 5 junge Reihen, die Gesammtzahl derselben somit 63 betragend. In den hintersten Reihen der Zunge fanden sich (jederseits) 85 Platten, und die Anzahl stieg weiter gegen hinten bis 90. Die Platten von schwach-gelblicher Farbe, denen der vorigen Art im Ganzen ziemlich ähnlich; die Höhe der äussersten meistens etwa 0,04–0,07 mm. betragend, die der Platten sich im Ganzen bis 0,14 mm. erhebend. Die äussersten (Fig. 9a) Platten niedriger und mit stärkerer Denticulation. Die Platten im Ganzen stärker und alle am Aussenrande und zwar stark denticulirt (Fig. 5–9), die innerste Platte mit einem starken Dentikel innen am Grunde des Hakens (Fig. 5aa.).

A comparison of these two descriptions shows the following differences: The rows of teeth in C. zebra are 95 to 100, in C. villafranca 63. The number of teeth in a row in C. zebra is 165 to 170, while in C. villafranca it is but 85 to 90. The form of the teeth as shown in Bergh's figures indicates clearly that the two forms belong to distinct species.

The oesophagus

The oesophagus of C. zebra leaves the pharynx at the dorso-posterior angle of the buccal mass. On each side, in a slightly dorsal position, the ducts from the single pair of salivary glands (fig. 2, *gl.sal.*) enter the oesophagus. These glands are long ribbon-like structures, folded along their mid-line and somewhat irregular on the margins. They are 3 cm. long and 2 mm. wide. From this point the oesophagus (*oes.*) continues posteriorly as a slender tube for 1 cm., passes through the nervous collar, and then expands into a thin-walled sac (15 mm. long by 5 mm. in diameter), the crop (*iglv.*). From the crop it continues, as a slightly broader tube (3 mm. in diameter), to the anterior portion of the liver mass, where it passes directly into the anterior end of the stomach (fig. 2, *ga.*). Throughout its entire length, the thin walls of the oesophagus bear longitudinal lamellae on their inner surface.

The stomach

The stomach is a large thin-walled sac, 30 mm. long by 15 mm. in diameter, lying within the anterior two-thirds of the liver mass. Its walls are slightly lamellated and perforated by innumerable large openings, the orifices of the evaginations which collectively constitute the 'liver' or digestive gland, so that apparently the cavity which we call the stomach is merely enclosed in a loose meshwork. Closely applied to the walls of the stomach are the ramifications of the nephridial organ (fig. 2, *ren.*).

The intestine

The intestine (fig. 2, *in.*) arises from the right side of the stomach about two-thirds the length of the stomach from its anterior end. This tube, at first rather narrow, has its greatest diameter (7 mm.) near its emergence from the stomach, and it gradually grows smaller as it nears the anal opening. From the stomach, the intestine passes anteriorly for about 25 mm. to the left side of the anterior end of the liver mass, where it turns abruptly to the right and runs diagonally crosswise and backwards for 10 mm.; then it bends again, continuing posteriorly for 40 mm., until it reaches the anal opening within the branchial circle. The walls bear longitudinal lamellae upon the interior, two especially conspicuous folds lying side by side upon the median ventral surface.

The liver

The liver occupies the posterior portion of the body cavity. It is a large pyriform organ, the broad anterior end being slightly bilobed in front. It is surrounded almost completely by the hermaphroditic gland, whose ramifications may be seen lying on the surface of the dark colored liver. In this species, the liver completely envelopes the stomach and the larger part of the kidney.

RENAL SYSTEM

It is impossible to determine anything very definite about the extent of the nephridial organ without careful histological study. However, it is possible to say that it consists of a main trunk with numerous lateral anastomosing ramifications, which cover the greater part of the dorsal surface of the stomach and probably run into the liver. A short ureter (fig. 2, *urt.*) leads from the kidney to the excretory orifice, a minute opening within the branchial circle close to the anal papilla. With the main trunk, where it merges into the ureter, is joined another duct (*ren.-pi'cr.dt.*), which arises from the pericardium, where a valvular enlargement allows the passage of fluid from the pericardium to the renal trunk.

RESPIRATORY AND CIRCULATORY SYSTEMS

These two closely related systems embrace the branchiae, the heart, the blood vessels and blood spaces, together with the blood gland. The heart lies within the pericardium on the upper surface of the posterior portion of the liver mass just anterior to the branchiae. It consists of an anterior muscular ventricle (fig. 4, *v.*) and a posterior thin-walled auricle (*aurl.*). From the apex of the ventricle a large blood vessel runs forward, giving off lateral branches to the liver and other organs. Just anterior to the liver mass occurs an enlargement, which sends one vessel (*va.sng.gen.*) to the genitalia, one to the blood gland (*gl.sng.*) and another (*va.sng.buc.*) which continues anteriorly to surround the buccal mass. Throughout the integument are numerous blood spaces, which lead to the posterior end of the body and pour their contents into the lateral blood vessels which enter the auricle at its posterior angles. It is probable that, as occurs in other forms, the entire primary body cavity surrounding the various organs is filled with the blood fluid and that this is collected and aerated in the branchiae, from which it passes directly into the middle portion of the auricle through the posterior median blood vessel of the auricle.

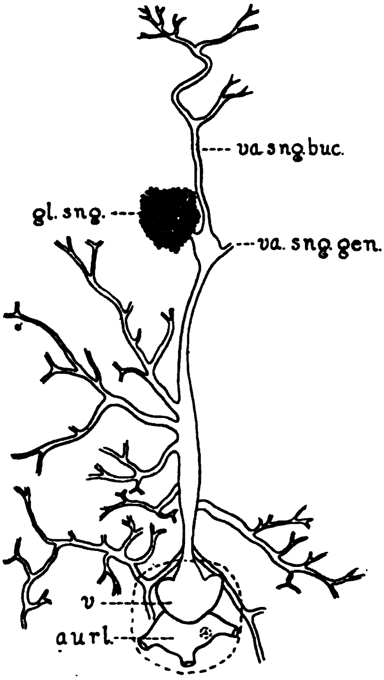

Fig. 4 Heart, blood gland and some of the more important blood vessels. *aurl.*, auricle; *gl.sng.*, blood gland; *v.*, ventricle; *va.sng.buc.*, blood vessel to buccal mass; *va.sng.gen.*, blood vessel to genitalia.

The blood gland (fig. 4, *gl.sng.*) is an irregular leaf-like mass made up of very small round cells. It lies immediately above the central nervous system and has an antero-posterior diameter of 7 mm. and a lateral diameter of 5 mm.

There are from 12 to 14 branchiae in the branchial circle. Each branchia is provided with an afferent and an efferent blood vessel, which carry the blood respectively to and from the branchiae.

NERVOUS SYSTEM

The nervous system consists of two main divisions—the central and the visceral or sympathetic part. The central part of the nervous system, enclosed within a semi-transparent membranous capsule, forms a nerve collar around the oesophagus, lying just beneath the blood gland. It is made up of three pairs of large ganglia,—two cerebral, two pleural and two pedal,—a small pair of buccal ganglia, and several minor ones.

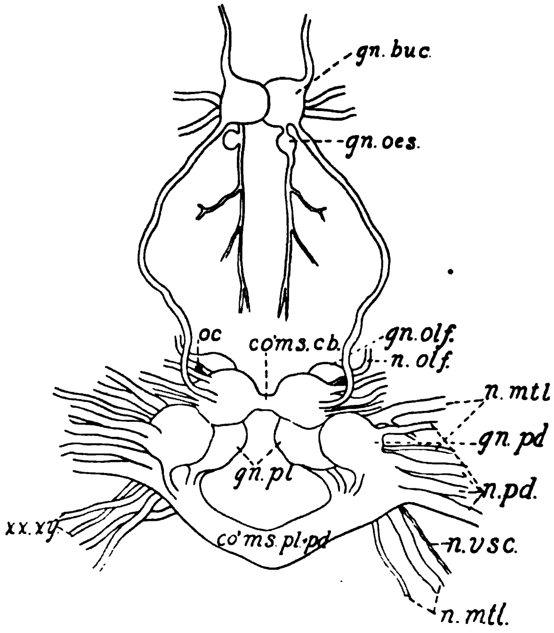

Fig. 5 Central nervous system seen from above and posteriorly. *co'ms.cb.*, cerebral commissure; *co'ms.pl.+pd.*, fused pleural and pedal commissures; *gn.buc.*, buccal ganglion; *gn. oes.*, oesophageal ganglion; *gn.olf.*, olfactory ganglion; *gn. pd.*, pedal ganglion; *gn.pl.*, pleural ganglia; *n.mtl.*, nerves to mantle; *n.olf.*, olfactory nerve; *n.pd.*, pedal nerves; *n.vsc.*, nerve to sympathetic system; *oc.*, eye; *xx*, *xy*, nerves to liver and genitalia.

The cerebral ganglia are the most anterior pair and are united with each other by a short (0.5 mm.) commissure (*co'ms.cb.*), as seen in figure 5. From the anterior margin of each projects the almost sessile olfactory ganglion (*gn.olf.*), which gradually tapers off into the large olfactory nerve, that passes into the base of the rhinophore. Upon the dorsal surface, near the external margin of the cerebral ganglia, arise the two small optic ganglia, each of which sends a short nerve to the corresponding eye (*oc.*). Each cerebral ganglion gives rise to three pairs of nerves, which pass forward and innervate respectively the oral tentacles, the mouth, and the lips. Besides these nerves, each ganglion sends from its mid-ventral surface a connective which passes around the oesophagus to the buccal ganglia (*gn.buc.*); these lie, side by side, ventral to the oesophagus just behind the buccal mass. A slender nerve leads forward from each buccal ganglion to the tongue. Two lateral nerves from each buccal ganglion innervate the buccal mass. Posteriorly, a small ganglion (*gn.oes.*) is joined to each buccal ganglion by an exceedingly short nerve. This oesophageal ganglion sends down the oesophagus a single nerve, which has three branches that spread over the tube.

Lying more dorsal and slightly posterior to the cerebral ganglia, to which they are joined by very short connectives, are the equally large pleural ganglia (*gn.pl.*). These generally give rise to three pairs of nerves which innervate the mantle. From one of them arises on the right side a slender nerve (*n.vsc.*), which communicates with the visceral part of the nervous system.

The pedal ganglia (fig. 5, *gn.pd.*), not so closely united to the others, lie in a more lateral position. From 4 to 5 pairs of nerves arise from them. Of these, two pairs of the smaller nerves innervate the body wall, while of the remaining larger ones at least two pairs pass to the foot as anterior and posterior pedal nerves.

The pedal and pleural commissures (*co'ms.pl.+pd.*) unite into a single broad band, which encircles the oesophagus ventrally, the nerve fibers arising in each case from the posterior margin of the ganglion. Two large nerves (*xx.xy.*), arising close to this commissure, innervate the liver and genitalia. Their precise origin is difficult to ascertain. The remaining pairs of nerves are distributed posteriorly to the mantle.

The study of the central nervous system of this nudibranch brought out the fact that there is considerable variation in the total enlarges to become continuous with the sheath of the penis, which, in turn, communicates with the genital atrium (*atr.gen.*).

The female genitalia are more complex. They may be roughly divided into two parts; the uterine or oviducal portion, and the portion specialized for the reception and storage of sperm, which is in direct communication with the oviduct. The oviduct leads from the bifurcation of the hermaphroditic duct directly to the albumen gland (*gl.albm.*), a large mass of closely convoluted tubules constituting the posterior portion of the anterior genital mass. From the albumen gland the duct passes forward into the mucous gland (*gl.muc.*), and terminates in the vagina (*vg.*), which is a single broad tube leading to the atrium. The mucous gland has a distinct duct (*dt.gl.muc.*) leading to the genital atrium. At its deep end the vagina expands into a rather large globular sac, which seems to be filled with detritus and a few free spermatozoa; this is the 'spermatotheca' of Bergh. About halfway between the external opening of the vagina and the spermatotheca a much smaller pear-shaped sac (*sp.cys.*) with thicker walls is connected with the vagina by a simple duct. This sac is the spermatocyst. It contains large numbers of spermatozoa. Just beneath this spermatocyst, on the ventral side of the vaginal tube, is the orifice through which the oviduct communicates with the vaginal portion of the female genitalia.

BIBLIOGRAPHY

BERGH, R. 1878 Untersuchung der Chromodoris elegans und villafranca. Malakozool. Blätter für 1878, pp. 1–36, Taf. 1 und 2.

1892 System der nudibranchiaten Gasteropoden. In Karl Semper's Reisen im Archipel der Philippinen, Theil 2, Bd. 2, Heft 13, pp. 993–1165. Also separately, Wiesbaden: Kreidel, 173 pp.

ELIOT, C. 1910 A monograph of the British Nudibranchiate Mollusca, Part viii (Supplementary). London, Ray Society, 198 pp., 8 pls.

HEILPRIN, A. 1889 The Bermuda Islands. Philadelphia [vi] + 231 pp., 17 pls.

SMALLWOOD, W. M. 1910 Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zool. Soc., London, Pt. I, pp. 137–145. (Contrib. Bermuda Biol. Sta. No. 18.)

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

VOL. 1.

1. COLE, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. GULICK, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. BOWDITCH, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. COE, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. BIGELOW, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
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7. BARBOUR, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
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10. BLACKMAN. M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pl. Feb., 1907.

VOL. 2.

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17. MARK, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
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23. GROSS, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. SMALLWOOD, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.
25. MARK, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48**( ) : 000–000.
26. SMALLWOOD, W. M., AND CLARK, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23**(4) : 625–636. Dec., 1912.

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3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903. Amer. Nat., **38**(451, 452) : 555–563. July-Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
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6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–348, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. Blackman. M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16) : 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2) : 189–238. 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate : 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1) : 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6) : 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7) : 217–226, 4 pl. July, 1911.
22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10** : 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.
25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48**( ) : 000–000.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23**(4) : 625–636. Dec., 1912.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
FOR RESEARCH.

No. 27.

THE STRUCTURE OF THE GORGONIAN CORAL PSEUDOPLEXAURA CRASSA WRIGHT AND STUDER.

By W. M. Chester.

With Four Plates.

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THE STRUCTURE OF THE GORGONIAN CORAL PSEUDOPLEXAURA CRASSA WRIGHT AND STUDER.[1]

By Wayland M. Chester.

Presented by E. L. Mark, March 12, 1913. Received April 5, 1913.

CONTENTS.

Introduction 737
Methods 740
General structure 740
Ectoderm 747
Mesogloea 751
Endoderm 754
Structures concerned in nutrition 757
Dorsal mesenterial filaments 759
Growth 760
Muscles and nerves 760
Skeleton and axis epithelium . 762
Summary 768
Bibliography 770
Explanation of plates . . . 773

INTRODUCTION.

PSEUDOPLEXAURA CRASSA is found on the reefs of Florida, of the West Indies, and of the Bermuda Islands. It is very abundant in the shallow water of the inner reefs of Bermuda, and is there one of the two or three very common sea whips; but it is found in the deeper waters of the outer reefs as well. The range in depth, to include the greater number of colonies, is from a position near the surface at low water to seven or eight meters.

Ellis and Solander (1786) described this colony under the name of Gorgonia crassa. Kölliker (1872) placed under the name of Plexaura branched, sea-rod forms in which the polyps completely retract into a comparatively thick coenenchyma, in which club-shaped and spiny spindle-shaped spicules appear. The different species were divided into two groups: Plexaura durae and Plexaura molles. Hargitt and Rogers (:01, p. 285) follow Verrill ('65, p. 34) in describing this form as Plexaura crassa. Wright and Studer ('89, p. 141–143), from observations of Bermuda specimens, created for this species a new genus, Pseudoplexaura. The new genus is characterized by them as follows: "axis horny, with a central calcareous portion, the outer layer of coenenchyme is soft and when dry friable; the inner layer contains a number of light purple or violet coloured irregularly stellate spicules or spindles with few rays." It is to be distinguished from Plexaura, in addition, by the following features, among others: colony feebly branched, older portions of horny axis solid, younger portions with calcareous particles in the center; polyps placed close together in an irregular spiral, completely retractile tentacles without spicules or having a circlet of them at their base; spicules mostly spiny spindles, with numerous pink stellate forms and a few club-shaped with attenuated foliaceous expansions.

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[1] Contributions from the Bermuda Biological Station for Research. No. 27.

The important characters of the colony are: the relative smallness of the spicules; spicules in the outer cortex, and irregularly stellate forms in the inner cortex; the massing of the latter to such an extent as to make the inner cortex firmer when dried, while the outer is friable; the absence of spicules in the tentacles and polyps; the sluggish but complete retraction of the polyps within the cortex; and the smooth cortex surface without projecting calyces in the contracted or dried colony. The polyps are numerous. When they are completely expanded the tentacles of adjacent polyps overlap, and the coenenchyme is hidden. Each tentacle has ten to twelve pairs of pinnae.

Of the three groups of alcyonarian corals,— Alcyonacea, Pennatulacea and Gorgonacea,— only representatives of the first and second have had their minute structure studied recently; the Gorgonacea, to which Pseudoplexaura belongs, have received little attention except from von Koch ('87) in his very important but early comparative study. Studies on the Alcyonacea have been relatively numerous. Von Koch ('82^a) described briefly the structure of Clavularia and other alcyonacean forms. Bourne ('95) described Heliopora coerulea and later made a very complete study of the origin and structure of its skeleton ('99). Ashworth ('99) studied the minute structure of Xenia Hicksonii Ash. and Heteroxenia elizabethae Köll. He found gland cells in the stomodaeum and correlated their presence there with the absence of the ventral and lateral mesenterial filaments. Hickson ('95) has given a detailed account of the cell structure of Alcyonium digitatum, and Pratt (:05) has described the digesting and mesogloea cells in several members of the Alcyonidae. She found a relatively large number of granular gland cells in the stomodaeum of feeding colonies and very few or none in starved ones. She held the mesogloeal network of cells to be neuro-phagocytic in function. By feeding with colored material, she proved the ingestion and the carriage of such material by the amoeboid movements of the mesogloeal cells. Kassianow (:08) reviewed the literature for the muscle and nerve systems in Alcyonaria digitatum, studied these systems and described in detail the cells of the ectoderm, endoderm and mesogloea with reference to them. He denied a nervous function for the neuro-mesogloeal cells of Pratt.

Among the Pennatulacea, studies have been made by Korotneff ('87) and by Bujor (:01) on Veretillum. They described the cells of the ectoderm and endoderm carefully.

The only complete study of the cell structure of representatives of the group Gorgonacea is by von Koch ('87), who made a comparative study of the structure and minute anatomy of the forms found in the Bay of Naples, giving most attention to Unicella (Gorgonia) cavolinii.

Wilson ('84) studied the mesenterial filaments of a number of species representing the three groups. He described the difference in structure of the ventral and dorsal mesenterial filaments and the origin of each from different germ layers.

Bourne ('99), in a paper giving the result of his study of the origin of the skeleton in Anthozoa, describes the origin and minute structure of the alcyonarian spicule and the structure of the massive skeleton of the alcyonarian Heliopora. He further studied the structure and origin of "holdfasts," or desmocytes, in Heliopora, as well as in madreporarian forms.

Woodland (:05) reviewed the literature on the origin of the alcyonarian spicule and made a very complete study of it for Alcyonium. The names of von Koch ('78, '82^b), Studer ('87, :06), and Alfred Schneider (:05) are important in the history of researches on the origin of the horny skeleton. Kinoshita (:10) has seen the origin of axis epithelium in young forms of Anthoplexaura and has confirmed von Koch's account of its ectodermal origin in the young form.

The study of this gorgonian coral (Pseudoplexaura crassa) was pursued during the summers of 1909 and 1910 at the Bermuda Biological Station for Research, and during the winter of 1909–1910 at the Zoölogical Laboratory of Harvard University. I wish to express my great indebtedness to Dr. E. L. Mark, the Director of these Laboratories for guidance and generous assistance.

Methods.

Small colonies were kept alive in large aquaria of running water for a short time. Small tips, 5 to 10 cm. long, were easily kept in smaller dishes of running water, if care was taken to keep them upright. Both Bouin's fluid and a five per cent formalin were successfully used as fixing agents. The fixing fluids were taken in some instances to the reefs and large tips or other pieces cut off from the colony and quickly transferred to the fluid; upon returning to the laboratory these were cut into small pieces. Both neutral formalin and Vom Rath's picric-osmic-acetic-platinic chloride fluid were used for nerve fixation. Corrosive sublimate and Wilson's fluid were both used, but did not give any better results than the formalin or Bouin's fluid. Decalcification was effected by 1 % acetic acid in absolute alcohol. Maceration by Hertwig's method gave good results. Delafield's haematoxylin and iron-alum haematoxylin could be used with decalcified material. Many sections of the soft tips were made without decalcification; but in these the haematoxylin overstained the spicules and the axial skeleton, obscuring the neighboring cells. By far the best general stain for these was Mallory's phospho-tungstic haematoxylin, which stained well after formalin and better after Bouin's fluid. This has the advantage that, while it does not obscure the spicules, it differentiates other structures.

General Structure.

A colony of Pseudoplexaura crassa presents a loosely divided group of long branches on a short stem; or the short stem may have short branches which divide and divide again, terminating in long whips. A drawing of a very young colony (Fig. A) illustrates the character of the branching. The short basal shaft spreads out on the coral rock or on old coral masses, secreting a skeleton that becomes very firmly attached to its substratum. The whips, which rise in many planes, are cylindrical, long and flexible, and taper somewhat gradually to the tips, which, however, end bluntly, or even with a slight enlargement. The brownish polyps stand at right angles to the branches (Plate 1, Fig. 1), closely crowded against one another, except at the tips of the branches, where the coenenchyma can often be seen even when all the polyps are expanded. Because the polyps when expanded stand at right angles to the branches, and are crowded, the colony in this condition looks like a miniature leafless shrub with unusually thick branches; because the tip is often bare of polyps or these are there contracted, the colony when seen at a distance below the surface may resemble very superficially a huge compound

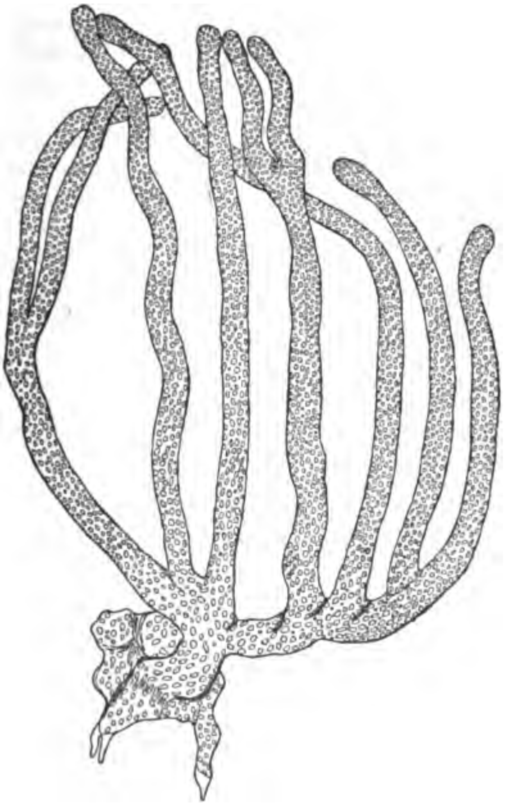

Fig. *A*. Young colony of Pseudoplexaura crassa, showing method of branching and the calycle openings in the coenenchyma.

tube sponge. But Figure *A*, although it shows the character of the branching, does not show the great number of branches, or whips, that occur in larger forms. As found on the Bermuda reefs, the species varies greatly in size. A small colony stood 90 cm. high, its terminal 60 branches spreading over a circle 60 cm. in diameter. Another colony was 110 cm. high with 250 terminal branches. A third measured 200 cm. in height and had 300 terminal branches. These three, taken from one place, are representative of the forms inside the outer reefs. On the outer reefs, forms 225 cm. high have been found. In the first colony (90 cm. high) the diameters of the stems were measured. Half way between the ground and the tips, with the polyps fully expanded, the branches were 4 cm. in diameter; at the base, 5 cm., and near the tip, a little more than 3 cm. After the polyps were fully contracted, the measurements were 1.5 cm. for the base, and .5 cm. for the tip.

A transverse section of a branch, or whip (Plate 1, Fig. 1), shows the structure. In common with that of the branches of Gorgonacea generally, there are recognizable three zones: a central axis of skeletal material (*ax.*), a fleshy enveloping layer,— the coenenchyma,— and a zone of polyps. The polyps can completely retract into spaces in the coenenchyma, whereupon two zones only are evident. The horny axis is very hard at the base but quite soft at the tip of the branches and, except at the basal end, is very flexible. To the naked eye, it is composed of two parts, a central, soft marrow, light in color (white in the figure), and an outer, harder, brown or black tubular shaft or cortex. The marrow has nearly the same thickness in all parts of the colony.[2] While its diameter is sometimes slightly smaller at the tip, it is not always so. Its variations are not wholly dependent on age, for it is slightly larger or smaller in parts of the stem and these parts occur irregularly. It is composed of a number of chambers filled with loosely branching threads (compare Plate 4, Fig. 58), and having walls of horny material, the chambers being generally arranged one above the other (Plate 4, Fig. 57, *med. ax.*). The loosely branching threads in the chambers are not shown in this figure, but are seen in the small chamber of the axis-cortex shown in Figure 58. The walls of the medullary chambers are very thin. Those of the axis-cortex chambers are very thin at the tip of a branch, while at the base they are very thick and hard. This is due to the fact that while the marrow chambers are laid down axially (i. e., at the end of the axis) in the branch, the cortex grows radially. The latter is composed of smaller chambers, which in longitudinal sections appear crescent shaped (Fig. 57, *ctx. ax.*). Not only is the cavity smaller, but the walls are thicker than those of the marrow. The first crescents laid down are adjacent to the marrow and very short, but as the axis-cortex increases in thickness, the outer ones are very long and have much thicker walls and thinner cavities. To the naked eye this gives the appearance of a solid cortex.

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[2] This cortex of the axis will be called axis-cortex to distinguish it from the more superficial coenenchymal cortex.

Where a branch is formed there is a break between the marrow of the branch and that of the stem (Plate 4, Fig. 61), and the axis-cortex forms a thick knee-like union at the stem for strength. At the base of the colony the axis-cortex is spread upon the substratum and the marrow is lacking. Many of the Bermuda colonies that were located on the shallow reefs, where the tips were exposed at the lowest tides, had lost the fleshy envelope of the tips of the branches for the distance of a few centimeters, the horny axis being here covered by diatoms. The fleshy coenenchyma was growing loosely around the old axis and taking the shape of the original branch. In such regenerated tips no new marrow was formed. In a few instances the old axis had been completely covered, but the coenosarc had not grown enough beyond the axis to give evidence of the presence or absence of a marrow distal to the old dead axis.

The coenenchyma is composed of two regions, an inner, exhibiting longitudinal canals, and an outer, containing the calycles or polyp-chambers. The longitudinal-canal region is characterized by a number of large canals running parallel to the axis (Plate 1, Fig. 1, *can. lg.*), and by the presence of purple, irregularly stellate spicules (Fig. *B*, 5–7) loosely massed in groups (*spc.'*). The spicules are represented diagrammatically in the left half of Figure 1, to show their positions. The longitudinal canals are less numerous at the tips of the branches — where there may be eight to ten — than they are at the bases, where twenty or more may be found. The diminution in number from the base to the tips is due to the running together of two adjacent canals or to the abrupt ending of one or more. Some of them run continuously from the basal half of the stem into the lower (abaxial) face of a branch. On the axial face of the branch they may be continuous with those of the stem, or the canal may begin abruptly in the branch without such connection. At the tips and on the branches the longitudinal canals connect with one another and with the polyp cavities by smaller canals (*can.*). At the base of the colony they run out radially and end blindly, or are connected with one another or with polyps by the smaller canals. At the tips the purple spicules are loosely arranged in two concentric cylinders separated from each other by the zone of canals. Where the branches are older, however, the inner cylinder breaks up into small groups, which lie between the axis and two adjacent canals (Fig. 1, *spc.'*); here the spicules interlock in a close mass. The diagrammatic nature of Figure 1 does not permit one to show the closeness of the interlocking of these spicules.

The outer region of the coenenchyma is a thick zone with polyp chambers (*cam. pyp.*), into which the polyps can completely retract. These chambers communicate with each other by a greatly branched system of small canals (*can.*). These canals are represented in only one half of Figure 1 (Plate 1). The actual canals are much more numerous and are more complexly branched than the diagram shows. Between the chambers white spindle-shaped and spiny spicules (Fig. *B*, 1–4), longer than the purple ones of the inner region, are found (*spc.*). Some of the deeper of these may be purple. Indeed there is considerable variation in both color and form of the spicules of different colonies and even of the same colony. These spicules have been described and figured by Wright and Studer ('89), by Hargitt and Rogers (:01) and by Verrill (:07). Figure *B* gives the relative proportions in size, as well as the differences in shape for the spicules of both the inner and outer regions of the coenenchyma. Figure *B*, 5–7, presents spicules of different shapes found near the long canals, where they are often locked together. In Figure *B*, 1, 2, are seen long white forms from the outer part, and in Figure *B*, 3, 4, spicules from the deeper part of that region.

The polyp-zone consists of the exposed part of the polyps or anthocodia (Fig. 1, *α*–*γ*). These when expanded are cylindrical. The mouth is oval and the eight tentacles of the crown stand at right angles to the column. The tentacles are conical, relatively long when fully expanded, and carry ten to twelve pairs of conical pinnae, arranged in two longitudinal lines, one on either side. No spicules are found in the polyps.

The anthocodia are brown and, when expanded, give the colony its prevailing color, which is caused by the presence of the Zooxanthellae (Plate 2, Figs. 20, 21, *zox.*) that crowd the endoderm cells and give a lighter or darker brown color in accordance with the degree of the animal's contraction. A little magnification shows that each disk is white and also that along the eight longitudinal lines corresponding to the union of the mesenteries with the body wall the color is white. The white appearance in both cases is due to the absence of these algae. The color of the coenenchyma between the anthocodia is distinctly white because of the absence of Zooxanthellae near the surface and the presence of white spicules.

The wall of the column of the anthocodium (Plate 1, Fig. 1, *β*) merges into that of the somewhat larger chamber in the coenenchyma and its endodermal epithelium is continuous with that of the chamber. The stomodaeum (Fig. 1, η, *stmd.*) is long, reaching when the polyp is expanded, nearly if not quite to the level of the surface of the coenenchyma; it is broadest in the dorso-ventral plane and has at its ventral angle a prominent siphonoglyph (Fig. 2, *sipy.*), which is not

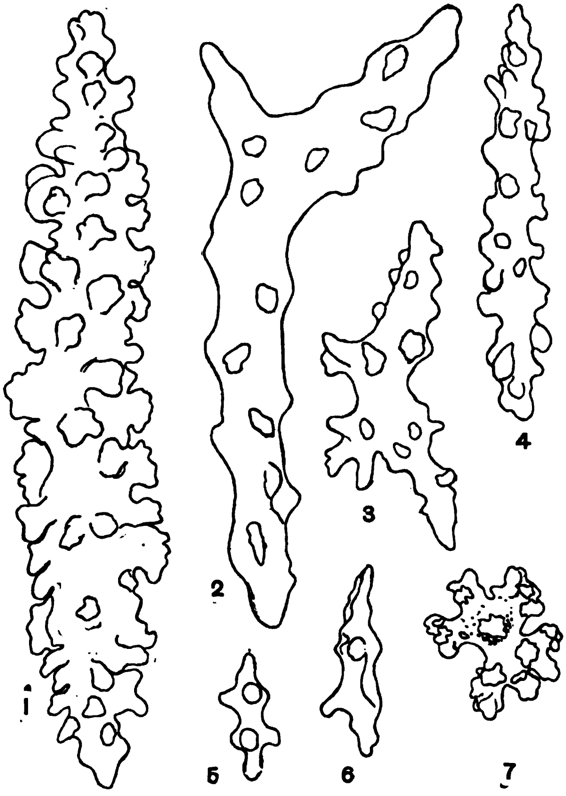


Fig. *B*. Calcareous spicules: × 90. 1–4, white spicules of outer cortex region; 5–7, purple spicules from the region of the longitudinal canals.

visible on the disk, but reaches nearly to the deep end of the stomodaeum. The structure is that usually found in the siphonoglyph of Alcyonaria. A transverse section through the stomodaeum is shown in Figure 2. The siphonoglyph is always ventral, and the longitudinal muscles of the eight mesenteries, as is usual in Alcyonaria, are located on the ventral sides of the mesenteries. Figure 3 shows a section made below the level of the first cut and near the level of the lower end of the stomodaeum — but still through the anthocodium — very close to the coenenchyma. The mesenterial filaments are cut in this section. The filaments belonging to the six ventral and lateral mesenteries are very short (never more than two millimeters long, and usually less than one), and appear as very slight thickenings of the edge of each mesentery. The filaments of the two dorsal mesenteries are seen to be deeply grooved; they are very long and pass with the mesentery from the stomodaeum to the very base of the chamber, being much convoluted at their lower ends. The section shown in Figure 4 is cut through the lowest part of the polyp cavity. The ventral and lateral mesenteries here have no filaments, but the dorsal filaments, cut across several times, show their grooved condition. In the lower region of the polyp chamber are also many ova, or else (the colonies being dioecious) masses of sperm mother cells covered by the endoderm. The ova are attached singly to the sides of the six ventral and lateral mesenteries. The eggs in July were large, but there was no evidence of fertilized eggs or of matured sperm.

Phases in the retraction of the polyp have been described by Wright and Studer ('89) and appear to be similar to those of Alcyonium (Hickson, '95). When the colonies were transferred from the conditions of the sea to those of the laboratory, it was seldom that they remained as fully expanded as at first. The polyps were appreciably shorter, but they had the shape and character described (Plate 1, Fig. 1, *a*). Sometimes the mouth was open and observation showed a slight current of water passing into the stomodaeum; at other times, or in other parts of the colony, the mouth was tightly closed. Sometimes the tentacles and pinnae were contracted to form eight short cones at the top of a column comparatively well expanded, the mouth being either open or closed. A circular constriction of a narrow region then appeared just below the disk, while the remaining part of the body bulged like a flask (Fig. 1, γ). A slow withdrawal of the polyp then occurred, evidently by means of the longitudinal muscles of the mesenteries, together with a slow inturning of the tentacles, until the disk and tentacles were at the level of the calycle. These now drew within and the oval or ovate calycle opening became visible (Fig. 1, δ). Sometimes in this method of contraction, the tentacles were first rolled toward the mouth, giving the disk and tentacles the appearance of a circle with eight indented radii (Fig. 1, β). The column was then contracted into the polyp cavity of the coenenchyma, as already outlined. But this method of contraction is not the invariable rule.

Sometimes, although the tentacles are contracted, the mouth remains open and the column is brought down nearly to the level of the coenenchyma, where it remains for a long time, the current of water to and from the stomodaeum evidently not being checked (Fig. 1, ϵ). Probably the coenenchyma is also capable of slight contraction and expansion, due principally to the action of the muscles of the mesenteries. When the tip of a branch is cut off, the coenosarc, with the polyps near the line of the cut, becomes appreciably of smaller diameter, thus partially covering the cut surface; and the same result is seen in the regenerating branches on the reefs, where the polyps have been turned from their radial position and are held in such a way as to lessen the area of the cut surface.

ECTODERM.

The ectoderm of the polyp wall in the expanded condition is more than one cell thick, showing an epithelial and a subepithelial part (Plate 2, Figs. 8, 18). In the epithelial part, the cover cells (*cl. teg.*) are conical, with a round or polygonal external surface as a base and with the opposite end, as apex, extending into the mesogloea as a process. In the expanded state of the polyp many of the cells are peltate or mushroom shaped, since they have the appearance of an external plate supported by a rapidly narrowing stem. This stem soon takes a thread-like character and may be branched (Figs. 9, 11). The outer surface is often convex, like a mushroom, but it may be indented, so that the section of it is cusp like (Fig. 18, *cl. teg.*). In the expanded condition of the polyp, the plate-like surface covers a relatively large area and the process becomes shorter; in the contracted condition, the cells approach a columnar shape, though the process is still thread-like at the mesogloeal end (Fig. 20, *cl. teg.*). The protoplasm is finely granular and sometimes large granules also occur (Figs. 10, 11). Although sometimes without vacuoles, these cells usually contain many small ones. The nuclei are large and granular and are situated in the outer half of the cells or near the point of the cusp. Among the covers cells are found nettle-cells or thread-cells with their nematocysts (Fig. 18, *nm'cys.*) and sense cells (*cl. sns.*).

The nematocysts are of two kinds. The larger and more numerous ones (Plate 2, Fig. 18 and Plate 4, Figs. 43–46) occur in batteries of six to ten, but they may be found in much larger numbers. The cyst is sharply outlined and is an elongated oval with a length three times its greatest width. Its length varies from 10 to 14 micra when unexploded. Around the cyst is a thin layer of granular protoplasm containing the flattened nucleus of the cell at the region of the greatest width of the cyst. The nucleus stains deeply with basic stains, the chromatin being abundant and granular. There is no trace of a cnidocil or of a cytoplasmic process projecting from the free surface of the cell. After staining in Mallory's phospho-tungstic acid haematoxylin or methylen blue a thick thread may be distinguished within the cyst, though not easily. It passes in a zigzag line from its attachment for a third of the length of the cyst and fills the remaining two thirds of the cyst in a tight coil (Fig. 44). When exploded the thread is quite thick and short and without barbs. Nematocysts of the second type (Fig. 47) are not found so abundantly. They are ovate and much smaller than those of the first type. The length is 5 to 8 micra. The nucleus is at the broad end of the enclosing cell. The thread with four or five spirals is sharply outlined even without stains. Exploded, it is long and slender, without barbs.

Sense cells (Plate 2, Figs. 8, 13, *cl. sns.*) occur among the cover cells, but are readily found only in the nematocyst batteries, where their bristle tips can be seen in favorable sections and where their nuclei reveal them by the size, which is smaller than that of the cover-cell nucleus. The sense cell is narrow and spindle-shaped with a long process extending into the mesogloea. Its protoplasm is slightly more evenly granular than that of the cover cells, though not greatly different from it; but its nucleus is small and round and can be readily distinguished from the larger nucleus of the cover cells. The bristle point is exposed between the cover cells, or between these and the nematocyst cells.

The subepithelial part of the ectoderm is made up of interstitial cells, globular cells with granules, nematocyst cells, and ganglion cells. It is not a sharply defined layer, since the cover cells and the sense-cell processes pass through it. The interstitial cells (Plate 2, Figs. 18, 20, *cl. in.*) are globular or have a central body with branching processes. In fact, the cells, or some of them, may change their shape in an amoeboid manner. The cytoplasm is similar to that of the cover cells; the nucleus may be smaller, though it varies greatly in size. The granular cells are filled with comparatively large granules and stain sharply with eosin. They have the appearance of the granular cells found in the mesogloea of the coenenchyma. Besides cells containing fully formed nematocysts, there are some which show stages in the formation of the nematocysts (Fig. 18, *cl. nm'cys.*).

Other cells, lying close to the mesogloea with processes parallel to it, are interpreted as ganglion cells (Fig. 20, *cl. gn.*). The difficulty with this interpretation is due to the presence in the adjoining mesogloea of the mesogloeal cells, which sometimes resemble the supposed ganglion cells in shape. In preserved material, the fibers of these ganglion cells sometimes possess many minute varicosities. The cytoplasm is more evenly and closely granular than in surrounding cells.

The ectoderm of the tentacles (Plate 2, Fig. 5) and pinnules (Fig. 6) is relatively thicker than that of the polyp wall, but shows the same superficial and deep layers. It also differs from that of the body wall in the smaller number of the large kind of nematocysts and in the presence of muscle cells in the ectoderm of the oral face of tentacle and pinnule. The deep end of the muscle cell (Figs. 16, 17) is elongated into a process which is perpendicular to the axis of the body of the cell, and runs lengthwise of the tentacle or pinnule. Each process contains in its axis a single highly refractive contractile fiber, or myoneme. The nucleus is small and finely granular. Usually the cell body is flattened in the plane of the muscle fiber; but the flattening may be at right angles to that plane. It is only occasionally that these muscle cells reach to the surface of the ectoderm and thus present the typical condition of an epithelio-muscle cell. Since all the muscle fibers run lengthwise of the tentacle, a transverse section of the tentacle (Fig. 7) shows, adjacent to the mesogloea of the oral surface and the sides, a very definite row of dots — the cut ends of muscle fibers. In sections dyed in Mallory's stain these fibers, being deeply colored, appear as dark dots, or, if cut somewhat obliquely, as short lines. In the partially contracted condition of the tentacle the dots no longer occupy a plane surface, for the originally plane surface is so folded that the line of dots is very sinuous, and even forms in some regions a series of pinnate figures.

The ectoderm of the polyp wall passes gradually into that of the coenenchyma, where, between the polyps, the cover cells (Plate 3, Figs. 23, 24) are very long and conical, but never show the indented peltate shape seen in the ectoderm of the polyps. The ectoderm of the coenenchyma shows a thicker subepithelial region, which gradually merges with the mesogloea. As a rule, the interstitial cells are so numerous in the thick mesogloea and the mesogloeal elements are so near the long cover cells that it is hard to find a boundary between the two layers. The nematocysts of the larger kind are arranged in very large and numerous batteries; those of the smaller kind occur individually and are very few. Both kinds of nematocysts have the same structure as those of the polyp wall. Sense cells, like those in the body wall and tentacles, are found in the batteries and increase in number with the increase in the number of cysts in the batteries.

Some of the ectoderm cells of the coenosarc between the polyps contain a prominent, highly refractive, homogenous fiber (Plate **3**, Figs. 24, 26, *fbr. sst.*), beginning near the nucleus and extending to the base of the cell, which is implanted in the mesogloea. The nature of these fibers is not perfectly certain, but it is probable that they are the same as the "Stützfasern" described by K. C. Schneider (:**02**, p. 622) for Anemonia; however, I have never seen evidence of their fibrilation, such as Schneider has shown to exist in the case of typical "Stützzellen." Figure 26 shows the distribution of these Stützfasern in the coenenchyma and their relation to the grooves which occur in these regions.

The ectoderm of the disk is, in its content and cell structure, like that of the oral face of the tentacle. When the disk is fully expanded, the stomodaeal epithelium reaches over on to the disk. The siphonoglyph and the dorso-lateral regions of the stomodaeum are both characterized by extremely long columnar ciliated cells. The siphonoglyph (Plate **3**, Fig. 32) has neither gland cells nor nematocysts, both of which are abundant in the other regions of the stomodaeum (Figs. 30, 31). The columnar cells of the siphonoglyph are long and of small caliber; each has a single strong cilium, or flagellum, which is longer than the cell. The cytoplasm is finely and rather densely granular, and each oval or elongated nucleus has one or two prominent nucleoli and a small number of chromatin granules. The nuclei occupy different levels in adjacent cells and are so situated that collectively they form a definite layer. Four layers, then, are recognizable in the ectoderm of the siphonoglyph (Fig. 32): (1) subnuclear, composed of the bases of the columnar cells, between which may be found occasional nutrition cells (*cl. nut.*) and ganglion cells, (2) the nuclear layer, (3) the layer between the nuclei and the basal granules of the cilia, (4) the cilia layer, a wide border characterized by the long and prominent basal granules. No ectodermal muscle cells are found in this region.

In the dorso-lateral portion of the stomodaeum (Plate **3**, Figs. 30, 31) four types of cells occur abundantly, supporting cells (*cl. sst.*), mucus cells (*cl. muc.*), granular gland cells (*cl. grn.*), and the small nematocyst cells (*cl. nm'cys.'*). The same four layers as in the siphonoglyph are found, but the nuclear zone is much wider, and the cell bases do not always terminate so sharply against the mesogloea. The cilia are short. The supporting cells (*cl. sst.* and Fig. 39, Plate **4**) are columnar, finely granular, with very small vacuoles, and with a short cilium that has a prominent, inverted cone shaped basal granule. The nuclei vary from oval to globular, but are more often oval, stain lightly, and have one or two prominent nucleoli. The mucus cells (Figs. 30, 37, *cl. muc.*) are columnar or flask like, not always reaching the full depth of the layer, sometimes staining evenly with eosin, at other times showing a loose network of protoplasm. This network stains deeply with muci-carmine. The nucleus is in the basal end, is globular or nearly so, and stains deeply. The granular digesting cells (*cl. grn.*) are very numerous in some colonies, few in others, and particularly few in starved colonies. They also are columnar or flask shaped with a small round nucleus, that stains lightly, near the middle of the cell. Large granules, staining deeply with haematoxylin, sometimes partly, sometimes completely fill the cytoplasm. Nematocyst cells of the smaller type (Fig. 31, *cl. nm'cys.'*) are abundant between the columnar cells in the cilia layer, but the cysts are smaller than those at the surface of the body. They are here nearly globular with a deeply staining nucleus at the lower end. The border, or cilia, region of the supporting cells (Fig. 39) sometimes shows the presence of variously shaped, but very small, digestive or nutritive granules. Below the nuclear layer and between the supporting cells are found many globular cells (Fig. 30, *cl. gl.'*), containing large granules that do not stain with haematoxylin. In a few cases these cells have shown karyokinetic figures. I interpret them to be young stages of gland cells and think they are the same as those described by Kassianow (:08). Ganglion cells are found not far from the mesogloea, but they are very few.

The dorsal filaments and the axis epithelium are described later in this paper.

MESOGLOEA.

The mesogloea is very thin in the body wall of the polyp and in the tentacles, as well as in the stomodaeum and mesenteries. The boundary between it and the ectoderm or endoderm is sharply marked wherever muscle fibers are found; where there are no muscle fibers the division is not clear. In the pinnules (Plate 2, Fig. 6) evidence of mesogloea is seldom found, and even in the tentacle (Fig. 5) cells are not often seen imbedded in it. In the stomodaeum the layer is made out with difficulty, but it is thicker than in the tentacles. In the mesenteries (Plate 3, Fig. 33) it is very evident, though thin, being sharply outlined in regions where there are muscles; but only occasionally, and then in the thicker parts of the layer, are there any included cells. A thin layer of it is also found between the endoderm of the mesenteries and the egg or the sphere of sperm mother cells. In the body wall, particularly at the base of the polyp (Fig. 19), it is thicker and here a few included cells appear. These cells (*cl. ms'gl.*) are smaller than the ectodermal cells and have a correspondingly small nucleus; they have several elongated, more or less branched processes. Cells in the coenosarc (Fig. 28, *cl. ms'gl.*) which I interpret to be the same as these are very numerous and show plate-like expansions of the terminal branches, which have been described by Kassianow (:08, p. 525) for Alcyonium. The mesogloea layer in the coenosarc is very thick (Fig. 22); here the newer mesogloea, that which is near the ectoderm, is less dense than the portion which occupies the deeper layers. Sections stained in either eosin or Mallory's phospho-tungstic haematoxylin show well the differences between these regions. The ectoderm cells of the coenosarc are very long and the interstitial cells at their bases very numerous. The latter have the appearance of being pushed away from the ectoderm as growth proceeds, and they arrange themselves, or are arranged, in masses or cords (Figs. 22, 28, *cl.cd.*), in which the individual cells are often only loosely associated. The interstitial cells (Fig. 24, *cl. in.*) which are still near the ectoderm, are globular or irregularly branched, but otherwise they are not different in appearance from the ectoderm cells of the coenosarc or of the polyp wall and tentacle; but they, together with the deeper and more specialized cells constituting the cords (Fig. 22), reach down even to the axis epithelium. A transverse section of such a cord is given in Figure 29; other sections are shown in Figures 27 and 28. In these cords some of the cells (*cl. in.*) are like the interstitial cells near the ectoderm. Others (*cl. grn.'*) contain few or many granules, which vary in size and staining capacity, but always stain, either slightly or heavily, in haematoxylin or in eosin. The granules vary in size in different cells. In other respects these cells resemble interstitial cells. Some of the cells at the edges of the cord are partially surrounded by the jelly of the coenosarc, and these are appreciably smaller and more elongated than the others. The loosely arranged cells of the cord show no extra-cellular matter except at the edges of the cord. Where such matter is evident, the characteristic finely granular mesogloea cells (*cl. ms'gl.*) are to be found with their greatly elongated processes and terminal branchings.

I believe the cells enumerated below constitute a genetic series:

(1) the interstitial cells (*cl. in.*), or some of them; (2) the loosely arranged cells (*cl. grn'.*) with few granules, and those with larger granules; (3) the cells on the outside of the cords, which are either partially or wholly surrounded by secreted matter; and (4) the smaller mesogloea cells (*cl. ms'gl.*); because the interstitial cells and the series of loosely arranged cord cells are continuous with each other. The cells of each series may change their shape by amoeboid movements, as sectioned living material has shown me. Any of the cells, except the mesogloeal cells, may have granules. The cytoplasm and the nuclei of all these cells are alike, except in regard to the size and shape of the cell and nucleus and the presence of granules. But it would not be correct to argue from this series of cells found in the coenosarc that the mesogloea is of exclusively ectodermal origin. The bases of the ectoderm cells seem active in the formation of mesogloea in all parts of the colony and there is evidence of the secretion of the same substance by cells associated with the axis epithelium in the axis region. The evidence drawn from Pseudoplexaura does not exclude the probability that the endoderm is also active in the formation of mesogloea in the tentacle, polyp wall, and particularly in the mesenteries. The small cells at the end of my series of four given above occur in most abundance where the jelly layer of the coenenchyma is most dense; they simply represent, it seems to me, the ultimate condition of cells whose usefulness may not be limited to the formation of mesogloea, but which in all of the earlier stages have been more or less active in the formation of such substance.

But not all of the mesogloeal cells belong in this series, nor are all those in the cords secreting cells. Some of the interstitial cells develop nematocysts and some are spicule-forming cells. Those forming nematocysts are very abundant in the coenosarc, and not only are stages in the development of the cyst found, but cysts (*nm'cys.*) as large and as fully formed as any near the surface are very abundant, not only among the interstitial cells near the ectoderm, but also in the cell cords and in the deep parts of the coenenchyma. These, I believe, have been carried in from the ectoderm by the rapid growth of the outer layer and by the amoeboid action of the cells around them.

In growing regions at the tip of the branches some of the interstitial cells, pushed deeper by growth, secrete the spicules, probably during special periods. The formation of the spicules is not different from that described by von Koch ('87), Bourne ('99), or Woodland (:05). The more or less rounded spicule-cell first shows a small calcareous mass (Plate 3, Fig. 23, *spc.*), which increases in size with the division of the cell and takes on a characteristic shape (Figs. 25, 28). Several nuclei are to be found in the cytoplasm enveloping most of the spicules, thus showing that the spicule-cell usually divides more than once. Figure 25 shows the arrangement of the organic matter of the spicule after decalcification; this is similar to the condition described by Bourne.

Certain of the cells in the mesogloea (Plate 4, Figs. 48–51) show ovoid or globular bodies similar to those described by Bourne ('99) and Woodland (:05) as possibly stages in nematocyst formation. Rounded nutritive cells also occur, sometimes few, but in the coenosarc of some colonies very abundantly. They are also found occasionally scattered among the ectoderm cells of the outer edge and also among endoderm cells, where they probably originate. They stain more deeply with eosin than the surrounding cells.

ENDODERM.

The endoderm lines the coelenteric side of the stomodaeum, the disk, the polyp wall, the mesenteries and all the canals. It is composed of three types of cells; supporting, mucus, and granular gland cells. Muscle cells are found in some parts. The cell characters are similar in the endodermal lining of the anthocodia (Plate 2, Fig. 20), the polyp chamber (Plate 4, Fig. 56) and the connecting canals (not including the long nutritive canals). The supporting cells are narrow and columnar, in contact with each other proximally and distally. In partially contracted individuals (Fig. 20) the supporting cells and the less numerous gland and mucus cells appear crowded into close contact; but in slightly expanded individuals (Figs. 55, 56) after fixation frequent spaces occur separating individual cells except at their two ends. The cytoplasm is coarsely vacuolated. A large nucleus is found somewhere in the basal two thirds of the cell. The cells are sometimes crowded with Zooxanthellae (*zox*), which are usually very numerous in and near the polyps, but are not so abundant in the deeper canals. Three, four, or more of these algae are common in the sections of each cell in the tentacles, polyp wall or outer coenosarc. Each endoderm cell has a single weak cilium inplanted in its free end, and at its attached end a myoneme, which runs circularly in the wall of the anthocodia and polyp chamber, and generally so in the canals. Mucus glands are abundant (*cl. muc.*); they appear as columnar cells with the cytoplasm in the form of a large-meshed reticulum that stains deeply with muci-carmine, and each has a darkly staining nucleus. There are occasionally found also gland cells (Fig. 55, *cl. grn.*) similar in character to the granular gland cells of the stomodaeum. The feeble staining of the nucleus and the presence of large granules in the cytoplasm show the likeness. The cells may be shorter or even spherical They are not limited to particular regions, but are scattered throughout the endoderm of the polyp wall, the canals and the mesenteries.

The endoderm of the polyp wall (Figs. 20, 55) has longer cells and stronger myonemes than that of the polyp chamber or canals. However, in the pinnules and in the tentacles, except at their very bases, the myonemes and the granular gland cells are lacking (Figs. 5, 6); otherwise the layer is here like that of the body wall.

The epithelial cells and the muscle elements of the endoderm of the mesenteries (Plate 3, Fig. 33) are specialized, at least in certain regions. On the so-called ventral surface of the mesentery the longitudinal myonemes belong to cells that are entirely below the free surface of the epithelium. These muscle cells are long and spindle-shaped (Plate 4, Fig. 38), with a small amount of cytoplasm enveloping the fiber (myoneme) and most abundant around the elongated nucleus. The cells are very numerous and the fibers are so arranged that in the cross section of the mesentery they form wavy rows of black dots adjacent to the ventral side of the mesogloea (Fig. 33, *my'nm.*). Other muscle fibers, that run radially on the mesentery, are found in both layers of the endoderm and their cells are epithelio-muscular, though in some cases the cell body may be slightly sunk below the surface. Where the endodermal epithelium covers the genital cells, the epithelial cells are shorter and bear no muscle fibers.

The endoderm of the longitudinal canals (Plate 4, Fig. 59) has very long cells as compared with that of the other canals; they are, however, of the same type, viz. supporting cells; they are slender, columnar, vacuolated, and slightly separated from one another except at their proximal and distal ends. No muscle cells, or myonemes, are found in this endoderm.

As compared with corresponding structures in other alcyonarians, it may be said, in brief, that the ectoderm of Pseudoplexaura is like that of the other members of this group described by previous authors, in having an epithelial and a subepithelial layer; in the shape and character of its cover cells it is like Alcyonium (Hickson, '95, Kassianow,

:08), and is not greatly different in its cell characters from Xenia (Ashworth, '99) or Veretillum (Buvor, :01). The nematocysts of the larger kind are about the size of those of Clavularia (von Koch, '82[a]), and are generally slightly larger than those of Xenia or Alcyonium. Those of the smaller kind seem significantly numerous in the stomodaeum. Sense cells like those of Alcyonium (Kassianow, :08) are associated with the nettle batteries; but the number of the batteries in the coenosarc and at the base of the polyps appears to be greater than in Xenia or Alcyonium, and the fewness of the nematocysts on the tentacles and pinnules seems unusual. Evidently the surface of the coenosarc between the polyps is an important region for the work that the large nematocysts do. The polyp often contracts to the level of the coenosarc with the mouth still open and the tentacles still spread, and I have seen food particles passed along from the coenosarc to the mouth by the tentacles. The nematocysts of the smaller sort are more evenly distributed on the outside of the colony, but they are very few. I failed to find gland cells on the outer surface, except perhaps in the coenosarc. Von Koch ('87) does not include them in his list of ectoderm cells for Gorgonacea, but they are present in representatives of the two other alcyonarian groups. It seems hardly probable that the slime which is given off by Pseudoplexaura when it is handled has come from the mucus cells of the endoderm. The fibers of certain of the ectoderm cells of the coenosarc (Plate 3, Fig. 24) are, as has already (p. 750) been suggested, possibly supporting fibers, such as K. C. Schneider (:02, p. 622, Fig. 510) has described for Anthozoa and other invertebrates, and figured for a sea anemone.

The mesogloea of the colony is very thin except in the coenosarc region; but here is thicker than that of the forms heretofore described, except the Gorgonacea.

The endoderm is similar to that of Alcyonium, and shows no significant features, except the absence of muscle fibers in the longitudinal canals. Menneking (:05), from the study of Stachodes and other forms, reached the conclusion that the longitudinal canals have originated as inter-mesenterial chambers of a terminal polyp. The absence of muscles in the walls of the longitudinal canals of Pseudoplexaura, in contrast with their presence in the mesenteries, together with the fact that the canals are sometimes traced to solenia without polyps, suggests that in this form the longitudinal canals have not originated in this way. Kinoshita (:10) did not succeed in finding muscle fibers in the endoderm of the longitudinal canals of the developing colonies of Anthoplexaura, but in the growing part he found solenia, which were sometimes continuations of the longitudinal canals. The structure of adult Pseudoplexaura supports the conclusion of Kinoshita, that the longitudinal canals have not always developed from inter-mesenterial chambers.

Structures Concerned in Nutrition.

Though I often experimented with small portions of a colony in the laboratory, I saw very little feeding. Plankton was given, but I saw none of it stunned, and only the smaller less active organisms, such as sea-urchin eggs, were swallowed. Sea-urchin eggs and small pieces of the flesh of fish were placed near the polyps and were often taken into the stomodaeum. Sometimes a polyp kept large pieces of sea-urchin ovary against its mouth for a long time. Usually the whole colony was quite fully expanded, except when it had been vigorously treated. On the reefs colonies with all the polyps contracted were very seldom seen. In the laboratory I could not find any difference in the condition of a colony at night and in the daytime in this respect. Individual expanded polyps may have the peristome closed, or polyps that are contracted so that the tentacles are spread out on the coenosarc may show it open; but there seems to be no special time for feeding. I think the food is undoubtedly from the plankton, and particularly the smaller and more sluggish forms.

The nettle cells of the smaller kind (Plate 2, Fig. 31, *cl. nm'cys.'*; Plate 4, Fig. 47) are very abundant in the ectoderm of the stomodaeum, while less numerous on the tentacles. Those of the larger kind (Plate 2, Fig. 26, *cl. nm'cys.*; Plate 4, Figs. 43–46) are most abundant in the coenosarc between the polyps and are seldom found in the tentacles. When sea-urchin eggs are scattered with a pipette over the tip of a branch whose polyps are expanded, they fall slowly, and do not seem to be stopped by tentacle or polyp, but collect in the grooves of the coenosarc. Associated with the falling of sluggish material on the coenosarc, adjacent individual polyps often contract down to the level of the coenosarc with the tentacles still partly spread and the mouth widely open. In such cases the eggs are often drawn into the current of the siphonoglyph. The supporting cells of the stomodaeum have at times small irregular granules at the distal end. These may be zymogen granules or, more probably, products of metabolism destined for other than enzyme use. Food material may be taken into these cells, and even algae have been found in them in a partially digested condition. Some of the food, then, is probably digested here, both in an intra-cellular and extra-cellular fashion. After the remaining food passes the stomodaeum it is in contact with the six ventral mesenterial filaments. These (Plate 4, Fig. 64) are very short thickenings of the margins of the six mesenteries, and occupy a position immediately below the stomodaeum. They are less than two millimeters long and in preserved material may be less than one. They begin at the deep end of the stomodaeum, but their gland cells may be found on the mesenteries a little above this. The cross section shows that this thickened margin is nearly cylindrical. The cells are mostly gland cells that are not different from the granular cells of the stomodaeum. A few supporting cells occur among the others and these may contain food matter. There are no nettle cells.

Until 1899, the stomodaeum was considered as merely a passage for the food, the mesenterial filaments being regarded as the only digestive organs. Wilson ('**84**) described the filaments of eleven genera from the three groups of Alcyonaria and concluded that the six lateral and ventral filaments are derived from endoderm and that the two dorsal ones are from ectoderm. The former contain gland cells and sometimes nettle cells, and are digestive in function; while the latter have two kinds of cells, are ciliated and are used for the production of currents. In 1899 Ashworth found mucous gland cells in the stomodaeum of Xenia, and correlated their presence with the absence of the ventral filaments. Miss Pratt (:**05**), by a very thorough and complete study of the feeding, in which she employed colored food, found that food was ingested, not only by the cells of the stomodaeum and filaments, but also by the mesogloeal cells. But no ingulfing of food was observed in the cells containing the granules. Gland cells were abundantly present in the stomodaeum of many members of the Alcyonaria, but the granular cells were met with in starved individuals only. Pseudoplexaura agrees with the forms studied by Pratt in the presence of gland cells in the stomodaeum and the abundance of the granular cells in the tips of individuals starved in filtered sea water. In Miss Pratt's experiments particles of fish artificially colored were also engulfed by stomodaeum cells, by the network of interstitial cells in the polyp wall, and by the mesogloeal cells near the outer surface of the coenosarc. Both the stomodaeum and the ventral filaments, then, are digestive structures; while the granular gland cells, which are quite abundantly scattered in the endoderm of the coelentera, including the canals and the mesenteries, may considerably aid in extra-cellular digestion.

Dorsal Mesenterial Filaments.

These filaments differ in origin, structure, and use from the ventral and lateral filaments. As a whole, the filament is a long, deeply grooved ribbon or cord, attached to the margin of the corresponding mesentery, and reaches from the stomodaeum to the depths of the polyp cavity; if there is a large basal canal connecting polyps with one another, it may even be continued into such canals. In cross section (Plate 4, Figs. 62, 63) the filament is much thicker than the mesentery and is deeply notched at its free margin. Consequently, in cross sections the mesogloea has the form of the letter Y. The epithelial cells occupying the space between the arms of the Y are of two kinds. The outer ones (*cl. fil.*), those nearer the ends of the arms of the Y, are the more numerous and are similar to the supporting cells of the stomodaeum. They are columnar, of small diameter and so closely packed that their nuclei are arranged in several rows. Each cell has a very strong cilium, and these cilia are so long that those of one side of the groove touch or cross those of the opposite side. The remaining cells, those occupying the base of the filament groove (*cl. fil.c.*), are few but larger, having broad bases and tapering slender necks. Their cell boundaries usually cannot be demonstrated. Near the base of each cell is a large, lightly staining nucleus. They possess no cilia. The cytoplasm is sometimes evenly granular, but often shows large vacuoles that stain with muci-carmine. The mucus, which they evidently have secreted, may sometimes be found between the cilia of the other cells.

Wilson ('84) has described in detail these dorsal filaments for other alcyonarians. But neither in his eleven genera, nor in the figures of Alcyonium by Hickson ('95), nor of Xenia by Ashworth ('99), are the cells represented to be as large and prominent as they are in Pseudoplexaura. I did not observe the effect of the presence of mucus in this groove, save that sometimes very minute particles, presumably of food, may be found in it; the mucus is probably for the purpose of catching material entering the polyp from another polyp or from the long canals: Portions of colonies kept for some days in the dark or in weak light lost their Zooxanthellae. The polyps of these portions with one another, since touching one does not result in a response from another. One can draw a pencil across a branch and get a contraction of the polyps only in that line, if he does not shake the branch. When a branch is shaken, all polyps begin to contract, although very slowly. I saw no reactions that would indicate taste as contrasted with touch. Food particles on the coenosarc cause the contraction of the polyps near it, the mouth and tentacles remaining expanded; but clean filter paper does the same. Neither in the field nor in the laboratory did I find muscular response to light. The polyps were expanded night and day alike.. In the laboratory, away from the sunlight they lost the Zooxanthellae and became white after a week's time.

SKELETON AND AXIS EPITHELIUM.

The structure of the axis skeleton has already been described under General Structure (p. 742). I find the axis epithelium (Plate 4, Figs. 54, 58, (*e'th. ax.*) always present and made up of two types of cells, the secreting cells and the holding cells, or desmocytes. The secreting cells are long and cylindrical or prismatic. Of the two ends, the one directed toward the skeletal axis may be designated as axis-end and the other as mesogloea-end; the former is flat, the latter tapers and is more or less rounded (Fig. 54). The large feebly staining nucleus is nearly in the middle, but typically somewhat closer to the axis-end of the cell. The cytoplasm is vacuolated at the mesogloea-end, but near the axis it is finely granular. This type of axis cell is always found at the tip of a branch, where the horny rim of the axis chambers is very thin; and I interpret this as a place of most active secretion. In any region of the colony, except at the very tip, some of the epithelial cells — sometimes only one, sometimes a comparatively large area of them — are modified into desmocytes (Plate 4, Fig. 41, *dsm'cy.*). These cells are broader than the secreting cells at the axis-end, and relatively shorter. At the axis-end they show a prominent border of striations perpendicular to the surface. These striations are due to slender rod-like differentiations of the cell, which seem to be the means by which the cells hold firmly to the axis, even when, in sections cut either free hand or after imbedding, the other cells are detached. Where the outline of the cell is complete (*dsm'cy.*), a nucleus like that of the secreting cells is present. Often, however, the cell has united with the mesogloea so that the boundary between the two is gone, and then the nucleus may have disappeared (*dsm'cy'.*). The axis-face of the cell (Figs. 41, 60) is usually flat, but may be concave (Fig. 58) or convex (Fig. 42). The desmocytes arise, or at least attain their differentiation, in the secreting epithelial layer. Cells in contact with the axis (Figs. 41, 58, *dsm'cy.*), that apparently are at first not different from the secreting cells, broaden their axis-end, pushing other cells away from the axis. To such a cell a mesogloeal process, probably secreted largely by adjacent epithelial cells, becomes applied, so that the cell then appears to be simply a prolongation of the mesogloea. The nucleus of the cell persists for a long time, but often it degenerates. Meantime the differentiation of the broad end of the cell shows it to be a desmocyte. Secretion on the part of the surrounding cells may continue around these desmocytes. Figure 42 shows that in this case much of the secreted layer of the axis was formed after the differentiation of the desmocyte and while it was still functioning as a hold fast. Figure 52 (*dsm'cy'.*), compared with Figure 42, shows evidence that the axial portion of the desmocyte may lose its connection with the mesogloea owing to the constriction of its neck by the formation of the horny secretion. It is in this way that some of the smaller chambers of the axis-cortex are formed. When this has taken place, other desmocytes appear in the same region peripheral to it.

In places where a great many desmocytes have been formed (Plate 4, Figs. 58, 60), the secreting cells are pushed back from the secreting surface in disarray. The displacement is perhaps a necessary result of the broadening of the ends of the desmocytes. At a later time, perhaps in response to the same stimulus that causes the beginning of a new skeletal chamber in places where desmocytes do not occur, such displaced secreting cells rearrange themselves preparatory to the secretion of a new lamella, leaving a lenticular space between themselves and the previously secreted portions of the axis. Later still, some sort of stimulus may then cause other desmocytes to appear among these secreting cells, probably as the result of the differentiation of a part of their own number. I consider these holding cells to be homologous to those seen by Fowler in a madreporarian coral and to those whose origin was described by Bourne ('99) for the madreporarians and for Heliopora, an alcyonarian with a calcareous skeleton; but I find no reference to similar cells for any other alcyonarian, except that possibly A. Schneider (:05, p. 128) found them; but if so, he evidently thought them artifacts. I have found them in all the colonies of Pseudoplexaura studied, and I have also seen them in the species of Euniceopsis and Gorgonia which are associated with them on the Bermuda reefs. In Pseudoplexaura the stimulus for the change from the secreting cell to the desmocyte must be irregular; it is not associated with any particular position of the polyps or with any structure that would give a regular pull or strain, since the cells occur sometimes in broad patches and sometimes singly; the latter are completely united with the mesogloea and are therefore fully formed. They remind strongly of the desmocytes described for the madreporarians by Bourne ('99), but there is no trace of the membrane which Bourne found between cell and axis. In their origin they also differ from those described by Bourne for Heliopora, where the stimulus for the striations occurred before the cells were in contact with the axis, to which they became adjacent secondarily; for in Pseudoplexaura the first trace of the striations is in cells already touching the axis. It should be noticed that in the present paper the desmocytes have been shown clinging to a horny skeleton, whereas previous researches have shown them only in connection with calcareous skeletons. Probably further study will show desmocytes present in a large number of alcyonarian forms.

The origin of the horny skeleton of the Gorgonacea has been the subject of much controversy, with which the names of von Koch, Studer, and A. Schneider have been prominently associated. A. Schneider (:05) has reviewed the literature carefully, and has shown that Ehrenberg, Dana, Milne-Edwards et Haime, and von Koch have maintained an ectodermal origin; while Lacaze-Duthiers, Kölliker, Studer, and Heider have not found the ectoderm involved. The arguments against the ectodermal origin, as summed up by Schneider and strengthened by his researches, have to do with (1) the presence of calcareous spicules within the horny skeleton, (2) the character of the union between the axis and its branches, (3) the existence of extra-axial horny masses in the cortex independent of epithelium, (4) the increase in size of the adult axis, (5) the embryonic origin of the skeleton.

Kölliker ('65, pp. 163–167) argued in part as follows: since the axis skeleton in certain forms (Mopsea) is composed exclusively of fused calcareous spicules, and since these spicules are not produced by epithelium, the skeleton is not an epithelial product. Studer ('87) and A. Schneider (:05) found numerous calcareous spicules in the axis, and thought the axis made up principally of them. I have found no evidence of such spicules in the axis of Pseudoplexaura, though I have found one or two instances of cellular matter that I conceive to have been included in the axis owing to the rearrangement of the secreting cells of the axis epithelium over a mass of desmocytes, and I can account for the possible enclosure of spicules in an axis in the same abnormal manner.

A. Schneider found that in Eunicella the axis of the branch (Nebenaxis) is at first separated from that of the stem, with which, however, it is later united. It is difficult to see how such an axis can be explained as the result of the secreting activity of an ectodermal epithelium, except in cases in which the branch is secondarily united (by anastomosis) to a stem, as in fan corals; but Eunicella does not usually have such a secondary union of branch and stem. Perhaps, however, the conditions in Eunicella are not essentially different from those which are met with in Pseudoplexaura, where I find that a sharp demarcation line between the axis of the branch and that of the stem also occurs (Plate 4, Fig. 61); here it is due to the fact that all branches of the axis are adventitious in respect to the marrow. At the region of branching, the marrow of the stem-axis is separated from that of the branch by the secreted cortex of the stem-axis. The walls of the marrow chambers in the branch were therefore formed after the axis-cortex of the main stem possessed an appreciable thickness (Fig. 61, *ctx. ax.*). But the existence of a stem-cortex between the marrow chambers of the stem and those of the branch is not inconsistent with an ectodermal origin of the epithelium secreting the axis of the branch, because axis-cortex is formed in the same manner as axis-marrow. In both cases the horny matter is laid down in the form of walls of chambers; and these differ only in the size and shape of the cavity and in the thickness of the wall. The chambers of the axis-cortex are smaller than those of the marrow, nevertheless they vary greatly among themselves in size (Fig. 57, *ctx. ax.*). It is assumable that, after some of these axis-cortex chambers of the stem had been formed (Fig. 61, *ctx. ax.*), other chambers with the characteristically thinner walls and larger cavities of the marrow, may have arisen at the place where a branch was about to be produced. The walls of these chambers would, then, be secreted by the same epithelium that recently had been building smaller chambers as an axis-cortex of the stem. The epithelial patch at the distal end of the axis of the branch would be composed of cells which had changed somewhat the character of their secretions, so that henceforth they would produce the larger thin-walled chambers characteristic of the marrow, whereas the remaining cells (at first situated in the periphery of this terminal patch) would continue to produce the smaller chambers, with thicker walls, such as they had been producing as axis-cortex of the stem; but now as the axis-cortex of the branch.

In no part of the axis of a stem or branch has the marrow grown in opposite directions, part distally, part proximally, for the walls of the chambers are always convex toward one end of the stem or branch — the distal end (Plate 4, Figs. 57, 61). The marrow must therefore have grown from the base of the branch toward the tip, just as it does in the stem from base toward tip, and not, as maintained by some writers, in the form of a separately established axis which grows in two directions: partly toward the tip and partly toward the stem to which, in their view, it is destined to be attached secondarily.

This type of axis — with the axis-cortex interposed between the marrow chambers of stem and branch — is the natural one for all axes except such as may have been formed by the dichotomous branching of a main stem. Such branches may possibly occur, but my dissections have not shown any. Occasionally, in colonies that had attained a height of ninety or more centimeters, the beginning of a branch was found on some of the whips. These, as short as five millimeters, had a soft axis that was continuous with the main axis and was formed of the characteristic marrow (Figs. 57, 61, *med. ax.*) and a very thin cortex. The marrow chambers were separated from those of the main stem by the cortex region of the stem-axis and, as has already been stated, were convex toward the free end of the branch, as in the main branch they were convex toward its free end. Although the earliest stage in the formation of the axis of a branch has not been seen in Pseudoplexaura, I am convinced that the axis skeleton of the whole colony in this species is not produced by a coalescence of separately established axes.

Pseudoplexaura gives no evidence on the third of Schneider's points, for no horny substance has been found in the coenenchyma. But the real issue between the two theories of ectodermal or non-ectodermal origin hinges on the results of observation as to the origin of the axis and as to the method of its subsequent growth; whether it is an epithelial secretion, as argued by von Koch ('78, '87), or results from a massing of mesogloeal material which is to be resorbed and replaced either by horny substance or horn and lime. Von Koch has described ('87) a larval stage of Eunicella a week old and has shown sections having the ectoderm continuous with the axis epithelium. His results have recently been confirmed by Kinoshita (:10) in embryos of Anthoplexaura. Kinoshita not only found the ectoderm of the pedal disk continuous with the axis epithelium, but he also has described and figured (Fig. 3–5) the beginning of the axis as a secretion product of the thickened ectoderm of the pedal disk; however, this primitive axis (a single case) did not by its upward growth push before it the floor of the digestive cavity of the primary polyp, but rather grew upward in the wall of the column at one side, so that the primary polyp had the appearance of being a lateral outgrowth from the axis. From this he concludes that the stem of the colony in Anthoplexaura apparently does not belong to the primary polyp, but to the coenosarc (at its base), just as in Pseudaxonia.

The existence of a secreting epithelium in the adult of Pseudoplexaura cannot be doubted. The axis-secreting cells are large, and this cell layer, which is evident, can be traced in free-hand sections from the tip of the axis to the spreading base near the substratum. I always find an unbroken axis epithelium around the tip. This seems to me to be irreconcilable with the method of growth outlined by Studer, and represented by the sections shown in A. Schneider's paper. For Studer's theory demands a mass of spicules at the tip, as well as in other places, perhaps,— spicules which are later to be resorbed. These spicules must of course develop in the mesogloea, and for their incorporation into the axis would require a break in the epithelium around the tip; but such a break I have not seen in Pseudoplexaura. There is often a massing of spicules at the tip *outside* of this epithelium, but there is no trace of their inclusion in the axis, nor of their conversion into it. The spicules are here pushed aside by the growth of the axis and remain as spicules in the mesogloea. A. Schneider holds that the axis epithelium as figured by von Koch, with which the epithelium of my figures is undoubtedly homologous, is the endodermal lining of the digestive cavity of the axial polyp, into which the axis has been pushed, and that the longitudinal canals are mesenterial chambers. But so far as regards Pseudoplexaura, the cells of the axis epithelium are not like endoderm cells. Moreover, the longitudinal canals vary considerably in numbers in Pseudoplexaura tips, being eight or *more*; besides, as Kinoshita found for Anthoplexaura, they have no muscles and sometimes end in solenia.

Pseudoplexaura, then, affords no evidence of spicules included in the axial skeleton; a secreting axis epithelium is present, the cells of which are unlike those of the endoderm in their arrangement and structure. Even when pushed aside by the spreading of the desmocytes, they are not easily to be mistaken for endoderm cells. Kinoshita's evidence in the embryos of Anthoplexaura is a strong support for the ectoderm theory. The results from the study of the adult of Pseudoplexaura are not in themselves complete evidence, but so far as they go speak strongly for the ectodermal origin of the horny axis, as indicated by von Koch.

SUMMARY.

Pseudoplexaura crassa, an alcyonarian of the group Gorgonacea, shows the character of Gorgonacea so far as regards the regions recognizable in cross sections of the branches; the branches have a central horny axis, a thick coenenchyma and an outer zone of polyps. The horny axis shows a marrow composed of large chambers arranged end to end, and a peripheral layer of smaller less regularly shaped ones arranged side by side and irregularly overlapping one another. The coenenchyma has, not far from the axis, a region of large longitudinal canals. These are sometimes prolonged at their tips into solenia. The polyps are long, and have ten to twelve pairs of pinnae on each of their tentacles. They are crowded, so that when expanded they hide the coenenchyma. Groups of small, crowded, irregularly stellate, purple spicules occupy the deeper parts of the coenenchyma, and larger, spiny and spindle-shaped, usually white spicules are in its outer part. No spicules are found in the polyps.

The ectoderm has the usual cover cells, nematocysts, sense cells, and interstitial, ganglion, and muscle cells. Small nematocysts are found in the ectoderm of the polyp's column, tentacles and stomodaeum. Large ones in considerable numbers are grouped into batteries in the coenosarc. Ganglion cells are very few, and muscle cells are found on the oral side of the tentacles and disk only. In the ectoderm of the coenosarc between the polyps some of the ectoderm cells have each a prominent supporting fiber, which runs from near the nucleus perpendicularly to the mesogloea.

The mesogloea is thin, except in the coenosarc regions, where it is very thick. Cords of cells like the interstitial cells of the ectoderm can be traced from the ectoderm to the deeper layers of the mesogloea. In these cords there are partly formed and fully formed nematocysts, spicule cells, and cells having an irregular shape and either containing granules or destitute of them. These irregularly shaped cells form a transition to the jelly-secreting cells, which are small and have many long branches. Large spicules are produced by characteristic secreting cells with large granules and one to many nuclei. Spheroidal nutrition cells occur in many colonies, but these are found in both ectoderm and endoderm; they probably originate in the endoderm of the canals which form a network through the mesogloea.

The endoderm cells are of characteristic form, being united with each other at the proximal and distal ends, but, in fixed material, separate elsewhere. In the tentacles, in the polyp wall and in many canals they contain large numbers of the alga Zooxanthella. Except in the tentacles and the longitudinal canals, they have myonemes running circularly. Unicellular mucus glands and granular cells, that are probably digestive in function, are numerous. The cells of the longitudinal canals differ from other endodermal cells in being much longer and in having no trace of myonemes.

Digestion is accomplished by cells of the stomodaeum, by the six ventral and lateral mesenterial filaments, and by scattered gland cells in the walls of the polyp cavity and the canals. The stomodaeum has, beside its supporting cells, mucus and granular gland cells. The mesenterial filaments, except the dorsal pair, are very short and their epithelium is composed of granular gland cells only, which give some evidence of intracellular digestion. I found no special feeding time and no regular alternation of contraction and expansion of polyps. Slow-moving organisms, which serve as food, are often transferred from the surface of the coenosarc between the polyps, where large nettle cells abound, to the mouth of a polyp that independently contracted to the level of the coenosarc with its mouth open. The two dorsal mesenterial filaments are very long and sinuous and their cell structure is peculiarly significant. The sides of the groove are lined by cells with strong cilia. The central cells, however, show the character of mucus cells and produce a mucous secretion.

The muscle system is similar to that of Alcyonium. The colony is characterized by the weakness of its responses and by the fewness of its nerve elements. The response to touch is not quick, and the coenosarc between the polyps is more sensitive than the polyps themselves.

The axis skeleton is surrounded by an epithelium consisting of elongated secreting cells, and in places, of desmocytes, or holding cells, these being shorter and wider, and exhibiting striations at the axial end. These cells become connected with the mesogloea secondarily. They may become isolated as the result of being completely enveloped in the secretion of horny material by the secreting cells. The desmocytes have already been described for Heliopora and for the madreporarians. The evidence in Pseudoplexaura favors an ectodermal origin of the axis skeleton.

BIBLIOGRAPHY.

Ashworth, J. H.

'99. The Structure of Xenia Hicksoni, nov. sp., with some Observations on Heteroxenia Elizabethae, Kölliker. Quart. Jour. Micr. Sci., vol. 42, pp. 245–304, pl. 23–27.

Bourne, G. C.

'95. On the Structure and Affinities of Heliopora coerulea, Pallas. With some Observations on the Structure of Xenia and Heteroxenia. Phil. Trans. Roy. Soc. London, vol. 186, pp. 455–483, pl. 10–13.

Bourne, G. C.

'99. Studies on the Structure and Formation of the Calcareous Skeleton of the Anthozoa. Quart. Jour. Micr. Sci., vol. 41, pp. 499–547, pl. 40–43.

Bujor, P.

:01. Sur l'organisation de la vérétille. Arch. de Zool. Exp. et Gen., Sér. 3, Tom. 9, Notes et Revue, No. 4, pp. xlix–lx, 7 fig.

Delage, Y., et Hérouard, E.

:01. Traité de Zoologie Concrète. Tom. 2, pt. 2, Paris. x + 848 p., 72 pl.

Hargitt, C. W., and Rogers, C. G.

:01. The Alcyonaria of Porto Rico. Bull. U. S. Fish Commission for 1900, vol. 20, part 2, pp. 265–287, 4 pl.

Hickson, S. J.

'95. The Anatomy of Alcyonium digitatum. Quart. Jour. Micr. Sci., vol. 37, pp. 343–388, pl. 36–39.

Kassianow, N.

:08. Untersuchungen über das Nervensystem der Alcyonaria. Zeit. f. wiss. Zool., Bd. 90, pp. 478–545, Taf. 29–31, 2 Textfig.

Kinoshita, K.

:10. Ueber die Postembryonale Entwicklung von Anthoplexaura dimorpha Kükenthal. Jour. Coll. Sci., Imp. Univ. Tokyo, vol. 27, art. 14, 13 p., 1 Taf., 3 Textfig.

Koch, G. von.

'78. Das Skelet der Alcyonarien. Morph. Jahrb., Bd. 4, pp. 447–479, Taf. 22, 23.

Koch, G. von.

'82^a. Anatomie der Clavularia prolifera n. sp. Morph. Jahrb., Bd. 7, pp. 467–487, Taf. 22, 23.

Koch, G. von.

'82^b. Vorläufige Mittheilungen über die Gorgonien (Alcyonaria axifera) von Neapel und über die Entwicklung der Gorgonia verrucosa. Mitth. Zool. Sta. Neapel, Bd. 3, pp. 537–550, 15 Textfig.

Koch, G. von.

'87. Die Gorgoniden des Golfes von Neapel und der angrenzenden Meeresabschnitte. Fauna u. Flora des Golfes von Neapel, Monogr. 15, Die Gorgoniden. x + 99 pp., 10 Taf.

Koch, G. von.

'91. Die Alcyonacea des Golfes von Neapel. Mitth. Zool. Sta. Neapel, Bd. 9, pp. 652–676, Taf. 25, 28 Textfig.

Kölliker, A. von.

'65. Icones Histologicae oder Atlas der vergleichenden Gewebelehre. Abt. 2, Leipzig, W. Engelmann. 4to, pp. 85–181, Taf. 10–19.

Korotneff, A.

'87. Zur Anatomie und Histologie des Veretillum. Zool. Anz., Jahrg. 10, pp. 387–390.

Krukenberg, C. F. W.

'87. Die nervösen Leitungsbahnen in dem Polypar der Alcyoniden. Vergl.-physiol. Studien, Reihe II. Abt. 4, Theil 1, pp. 59–76, Taf. 1. Heidelberg.

Menneking, F.

:05. Ueber die Anordnung der Schuppen und das Kanalsystem bei Stachodes ambigua (Stud.), Caligorgia flabellum (Ehrbg.), Calyptrophora Agassizii (Stud.), Amphilaphis abietina (Stud.) und Thouarella variabilis (Stud.). Arch. f. Naturg., Jahrg. 71, Bd. 1, pp. 245–266, Taf. 8, 9.

Pratt, Edith M.

:05. The Digestive Organs of the Alcyonaria and their Relation to the Mesogloeal Cell Plexus. Quart. Jour. Micr. Sci., vol. 49, pp. 327–362, pl. 20–22.

Schneider, A.

:05. Das Aschenskelet der Gorgoniden. Arch. f. Naturg., Jahrg. 71, Bd. 1, pp. 105–134, Taf. 5, 6.

Schneider, K. C.

:02. Lehrbuch der vergleichenden Histologie der Tiere. Jena. G. Fischer, xiv + 988 p., 691 Fig.

Studer, T.

'87. Versuch eines Systemes der Alcyonaria. Arch. f. Naturg., Jahrg. 53, Bd. 1, pp. 1–74, Taf. 1.

Studer, T.

'06. Ueber die morphologische Bedeutung der Achse der Gorgonacea. Verh. allgem. schweiz. naturf. Ges., Versam. 88, pp. 52, 53.

Verrill, A. E.

'65. List of the Polyps and Corals sent by the Museum of Comparative Zoölogy to other Institutions in Exchange, with Annotations. Bull. Mus. Comp. Zoöl., vol. 1, no. 3, pp. 29–70.

Verrill, A. E.

:07. The Bermuda Islands, Part 5, An Account of the Coral Reefs. Trans. Conn. Acad. Sci., vol. 12, pp. 204–348, pl. 28–40. (Alcyonaria, pp. 296–317.)

Wilson, E. B.

'84. The mesenterial Filaments of the Alcyonaria. Mitth. Zool. Sta. Neapel, Bd. 5, pp. 1–27, pl. 1, 2.

Woodland, W.

:05. Studies in Spicule Formation. II.— Spicule Formation in Alcyonium digitatum; with Remarks on the Histology. Quart. Jour. Micr. Sci., vol. 49, pp. 283–304, pl. 16, 17.

Wright, E. P., and Studer, T.

'89. Report on the Alcyonaria collected by H. M. S. Challenger during the Years 1873 to 1876. Report on the Scientific Results of the Exploring Voyage of H. M. S. Challenger, Zool., vol. 31, lxxii + 314 p., 43 pl.

EXPLANATION OF PLATES.

Abbreviations.

ax.	axis.
cam. pyp.	polyp chamber.
can.	canal.
can. lg.	longitudinal or nutritive canal.
cl. cd.	cords of cells in mesogloea.
cl. fil.	ciliated cells of the mesenterial filament.
cl. fil. c.	central cells of mesenterial filament.
cl. gl.	gland cell.
cl. gl.'	incipient gland cell of stomodaeum.
cl. gn.	ganglion cell.
cl. grn.	granular digesting cell.
cl. grn.'	granular cell of mesogloea.
cl. in.	interstitial cell.
cl. ms'gl.	mesogloeal cell.
cl. muc.	mucus cell.
cl. nm'cys.	nematocyst cell, large kind.
cl. nm'cys.'	nematocyst cell, small kind.
cl. nut.	nutrition cell.
cl. sns.	sense cell.
cl. spc.	spicule-producing cell.
cl. sst.	supporting cell.
cl. teg.	cover cell.
ctx. ax.	cortex of axis skeleton.
dsm'cy.	desmocyte.
dsm'cy'.	desmocyte, showing union with mesogloea.
ec'drm.	ectoderm.
en'drm.	endoderm.
e'th. ax.	axis epithelium.
fbr. sst.	supporting fiber.
fil. d.	dorsal mesenterial filament.
fil. v.	ventral (or lateral) mesenterial filament.
med. ax.	marrow of axis skeleton.
ms'enr. d.	dorsal mesentery.
ms'enr. v.	ventral mesentery.
ms'gl.	mesogloea.
my'nm.	myoneme.
nm'cys.	large kind of nematocyst.
nm'cys'.	small kind of nematocyst.
or.	oral.
ov.	ovum.
sipy.	siphonoglyph.
spc.	spicule.
spc'.	spicule of outer coenenchyma.
stmd.	stomodaeum.
zox.	zooxanthella.

Figures.

All drawings, except Figures 1 and 61, were carefully outlined with a camera lucida, and the details filled in afterwards. The magnification (except in Figures 1 and 61) is 675 diameters, unless otherwise indicated in the description of the Figure.

PLATE 1.

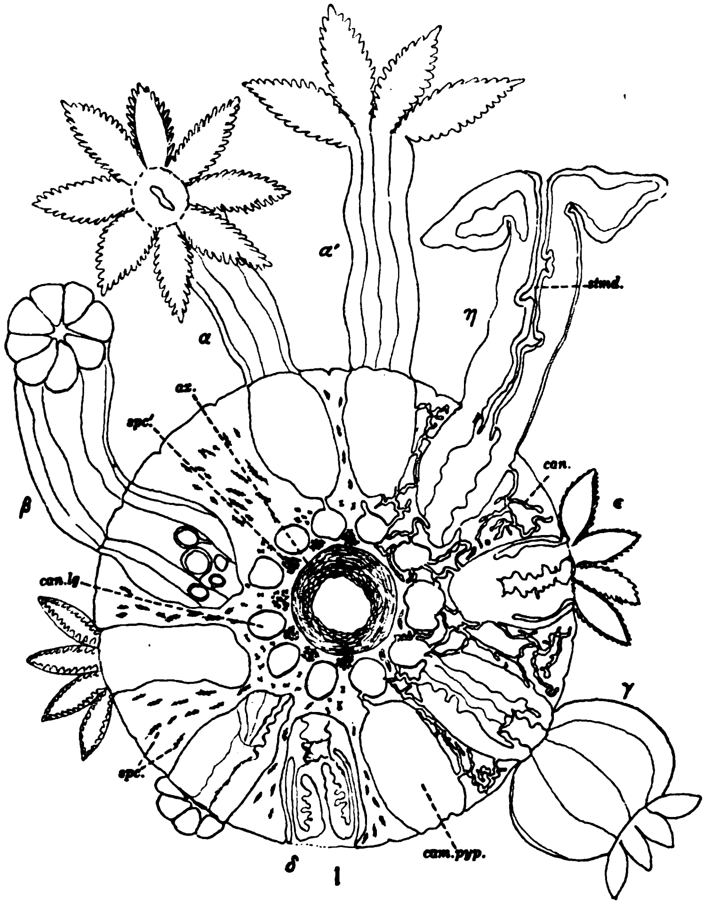
Fig. 1. Diagram of a transverse section through a branch of a colony, showing polyps in various stages of retraction.

Fig. 2. Transverse section of a polyp through the stomodaeum. × 15.

- *α*, expanded polyp, oral aspect
- *α′*, expanded polyp, seen from the side
- *β*, expanded polyp with inrolled tentacles
- *γ*, polyp partially retracted
- *δ*, polyp completely retracted
- *ε*, polyp with column retracted, but with expanded tentacles and with open mouth
- *η*, polyp in longitudinal section

Fig. 3. Transverse section of a polyp just below the stomodaeum. × 15.

Fig. 4. Transverse section of a polyp through the lower polyp cavity. × 15.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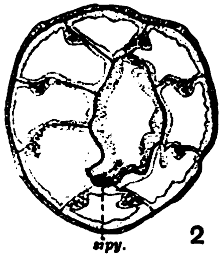
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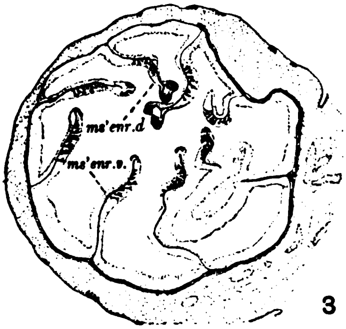
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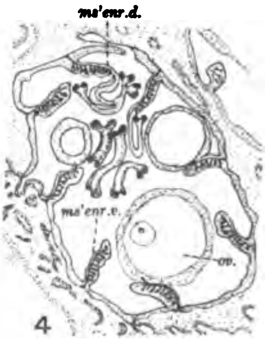
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PLATE 2.

Fig. 5. Longitudinal section from the oral wall of a tentacle.

Fig. 6. Transverse section of pinnule. × 600.

Fig. 7. Transverse section of a tentacle, showing the arrangement of the muscles. × 85.

Fig. 8. Longitudinal section of ectoderm of polyp wall.

Figs. 9–12. Isolated ectoderm cells of polyp wall, drawn from maceration preparations.

Figs. 13–17. Ectoderm cells of tentacle isolated by maceration.

Fig. 13. Two cover cells and a sense cell.

Figs. 14, 15. Interstitial cells.

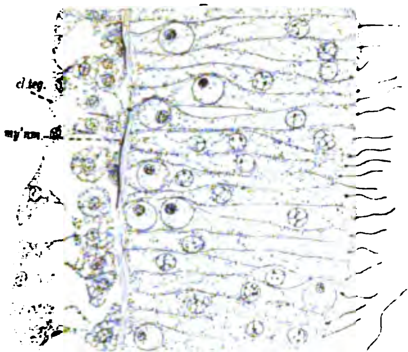
Figs. 16, 17. Muscle cells of the ectoderm.

Fig. 18. Longitudinal section of the ectoderm of polyp wall, in state of expansion.

Fig. 19. Section of ectoderm of the body wall of a polyp near the coenosarc.

Fig. 20. Longitudinal section of polyp wall, partly contracted. × 600.

Fig. 21. Longitudinal section of tentacle wall, aboral side. × 600.



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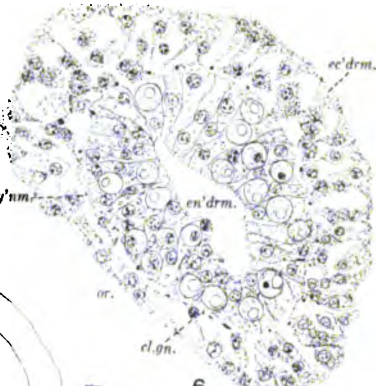

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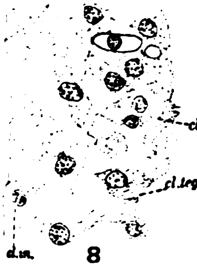

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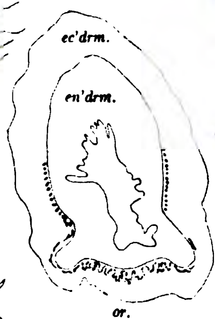

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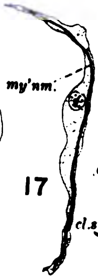

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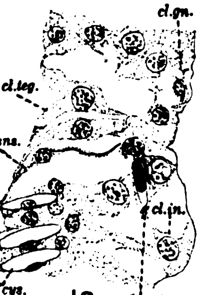

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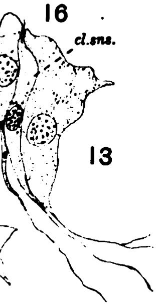

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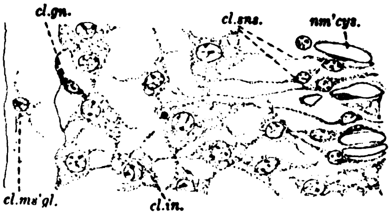

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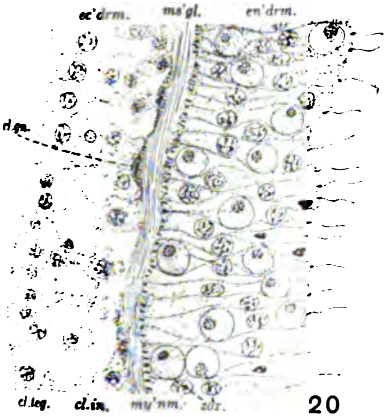

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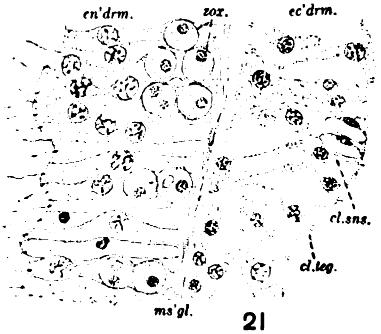

21

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PLATE 8.

Fig. 22. Section through the coenosarc to show cords of interstitial cells. × 67.

Fig. 23. Section of epidermis of coenosarc.

Fig. 24. Ectoderm of coenosarc in the region shown in Figure 26.

Fig. 25. Section of decalcified spicule, showing spicule-producing cells.

Fig. 26. Outline of portion of coenosarc between polyps, to show the distribution of supporting fibers and of nettle batteries. × 67.

Fig. 27. Section to show mesogloea.

Fig. 28. Section showing mesogloea cells. × 400.

Fig. 29. Section of "a cord" in mesogloea of coenosarc.

Fig. 30. Longitudinal section of epithelium (ectodermal) lining the stomodaeum and of mesogloea.

Fig. 31. Longitudinal section of the wall of stomodaeum, showing both ectoderm and mesogloea.

Fig. 32. Transverse section of a part of siphonoglyph.

Fig. 33. Transverse section of a portion of a mesentery.

CHESTER–PSEUDOPLEXAURA **PLATE 3**

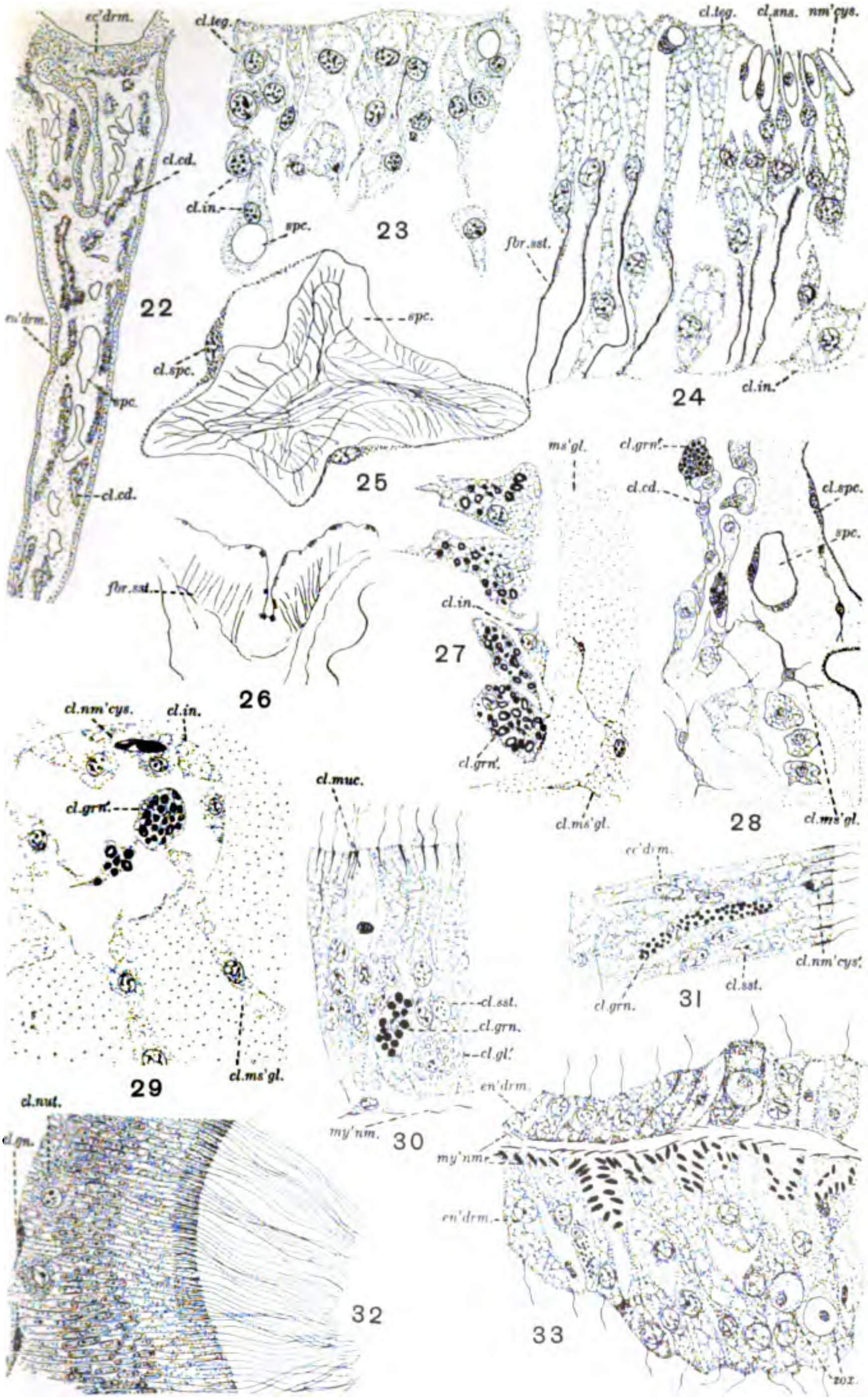

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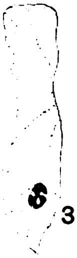
PLATE 4.

Figs. 34–37. Cells isolated by maceration.
Fig. 34. Gland cell of endoderm.
Figs. 35, 36. Supporting cells of endoderm.
Fig. 37. Mucus cell of stomodaeum.
Fig. 38. Muscle cells of mesentery.
Fig. 39. Supporting cell of stomodaeum.
Fig. 40. Granular gland cell of stomodaeum.
Figs. 41, 42. Axis epithelium, with desmocytes.
Fig. 43. Large kind of nematocyst cell, partly exploded.
Fig. 44. Large kind of nematocyst cell, unexploded.
Figs. 45, 46. Large kind of nematocyst cell exploded.
Fig. 47. Small kind of nematocyst cell.
Figs. 48–51. Interstitial cells containing ovoid bodies.
Fig. 52. Desmocyte enveloped in horny matter.
Fig. 53. Face view of the striated ends of six desmocytes.
Fig. 54. Secreting cells of axis epithelium.
Fig. 55. Longitudinal section of endoderm of polyp wall in the region of the column.
Fig. 56. Longitudinal section of endoderm of a polyp chamber.
Fig. 57. Longitudinal section of the axial skeleton, near the tip of a branch.
Fig. 58. Section of a portion of axis with its axis epithelium and desmocytes.
Fig. 59. Longitudinal section through the endoderm of a longitudinal canal.
Fig. 60. Epithelium of axial skeleton, with desmocytes.
Fig. 61. Diagram of a longitudinal section of the axial skeleton to show the nature of the skeleton in the region of a branch.
Fig. 62, 63. Cross sections of two dorsal mesenterial filaments.
Fig. 64. Cross section of a ventral mesenterial filament.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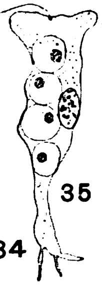
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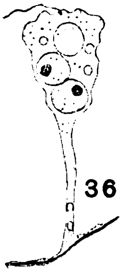
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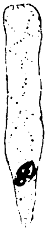
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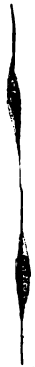
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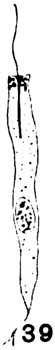
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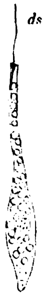
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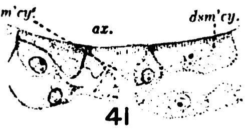

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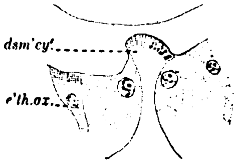

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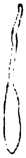
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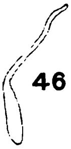
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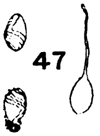
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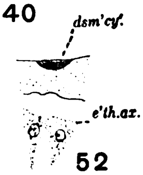

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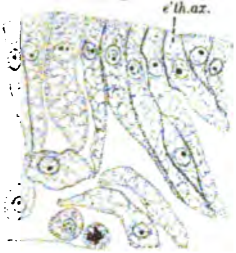

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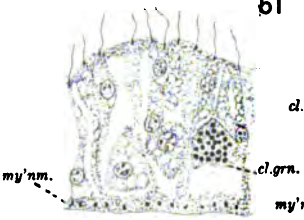

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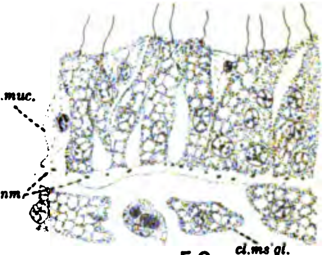

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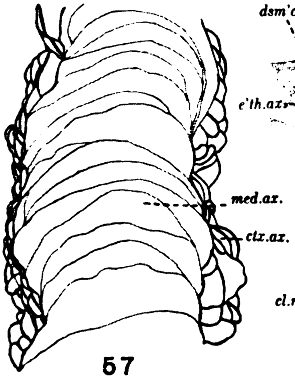

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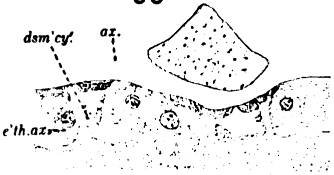

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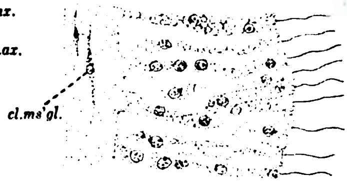

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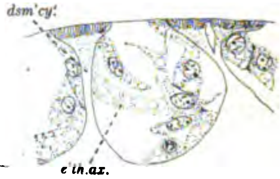

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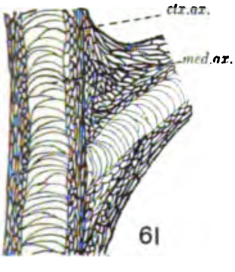

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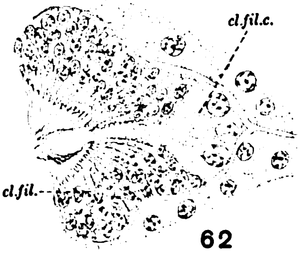

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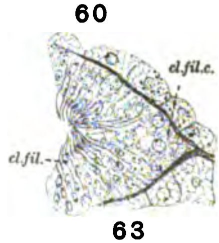

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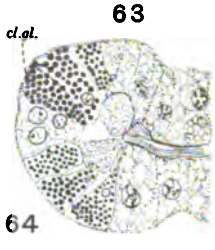

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

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2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
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7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908-
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20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pl. July, 1911.
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26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23**(4): 625–636. Dec., 1912.
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STUDIES ON THE PERIPHERAL NERVOUS SYSTEM OF AMPHIOXUS.

By Harriett Lehmann Kutchin.

With Eight Plates.

From the Proceedings of the American Academy of Arts and Sciences,
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October, 1913.

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BY HARRIET LEHMANN KUTCHIN.

Presented by E. L. Mark. Received June 2, 1913.

TABLE OF CONTENTS.

Page
Introduction 571
Literature 572
Material and Methods 572
Dorsal nerves 574
    A. Nerves of the rostrum 574
    B. Nerves of the buccal region 579
    C. Nerves of the velum 589
    D. Nerves of the branchial region 593
    E. Nerves posterior to the atriopore 603
    F. Spinal ganglia 606
    G. Structure of the dorsal nerves 607
    H. Sensory endings of dorsal nerves 608
Ventral nerves 615
    Bibliography 621
    Explanation of figures 626

INTRODUCTION.

THE literature on the nervous system of Amphioxus presents such divergence of opinion that it is probable many facts with regard to the structure, distribution and functions of this system still remain to be determined and illustrated. It will be impossible to more than theorize as to the primitiveness or the degeneracy of the nervous system of Amphioxus until the main features of its organization are more clearly understood. The present research aims to contribute toward a knowledge of the structure and distribution of the peripheral nerves, and it is hoped may aid in furnishing a basis for comparison with other vertebrates.

The following studies were undertaken in 1904 by the writer while holding the Alice Freeman Palmer Fellowship of Wellesley College.

Contributions from the Bermuda Biological Station for Research. No. 28.

I wish to express my sincere thanks to that institution for the exceptional opportunities afforded by the fellowship. Professor E. L. Mark of Harvard University suggested this field for investigation, and I am also indebted to him for kindly advice and criticism. The work was carried on in 1904–1905 at the Bermuda Biological Station and in the Harvard Zoölogical Laboratories while I was registered as a Graduate Student in Radcliffe College. In the spring of 1905 the problem was continued at the Naples Zoölogical Station, through the kindness of the "Association for Maintaining the American Women's Table at the Zoölogical Station at Naples and for Promoting Scientific Research by Women." I am under obligation to those in charge of the Naples Station for many courtesies extended throughout my stay. During the year 1905–1906 laboratory privileges were kindly afforded me for further work in the Zoölogical Laboratories of the University of Chicago.

Two species have been used as a basis for study, the Caribbean Amphioxus, Branchiostoma caribaeum, found in Bermuda waters, and the form so abundant at Naples, Branchiostoma lanceolatum.

LITERATURE.

Retzius (**'91**) and Dogiel (**:02**) have reviewed the literature on the peripheral nervous system of Amphioxus in considerable detail, presenting particularly points where lack of agrreement exists. Other authors, as Rohde (**'88**) and Heymans et van der Stricht (**'98**), give historical accounts in connection with their personal observations. It is therefore unnecessary to give here a connected discussion of the literature; the work of the several authors will be taken up in connection with each question in its bearing upon the descriptive part of this paper. An extensive bibliography of Leptocardii is given by Lönnberg (**:01**—, pp. 206–214).

MATERIAL AND METHODS.

At the Bermuda Biological Station the tissues of a large number of Branchiostoma caribaeum were impregnated with methylene blue by the *intra-vitam* method. Gold chloride and the methods of Golgi were also extensively used. Many variations were employed in the use of all these methods, with the view of bringing out details of structure, and these methods were found to give excellent results. Material was also hardened in various fixing fluids, 10% formol proving to be particularly valuable. The same methods were employed at Naples upon Branchiostoma lanceolatum, but a larger number of preparations was made, to provide against the uncertainty of impregnation. Further discussion of these methods will be taken up in the body of this paper, but a few general remarks are in place here.

The best impregnations of both superficial and visceral nerves were obtained by immersion of the living animals in sea-water colored a moderately dark blue with a stock mixture of ½% to 1% of methylene blue in normal salt solution. The sea-water mixture should never be opaque. It is probable that the small quantity of salt in the mixture causes the epithelium to loosen, thus permitting more direct action of the methylene blue. Such preparations are not, of course, suitable for study of sensory endings in the skin. Specimens immersed in this mixture do not appear impaired in vigor at the end of two or three hours. The subsequent exposure to air recommended by Dogiel (:02) is of great importance; the length of time required for different nerves can be determined only by continued observation under the microscope. The specimen must be kept moist with the methylene-blue mixture during such exposure. Fixation was usually effected by the ammonium-picrate method, and it was found that the addition of a few drops of 1% osmic acid to each 100 cc. of the ammonium picrate used for fixing these preparations greatly aided in their preservation. Material treated in this way, preserved in the usual ammonium-picrate and glycerine mixture, and carefully guarded against unnecessary exposure to light, was found in excellent condition at the end of two years, while other preparations fixed without osmic acid were practically useless after one year.

The gold-chloride method recommended by Hardesty (:02) for use after fixation with 10% formol proved useful in demonstrating motor fibers and their endings. I regard this method as worthy of wider use because of its accurate fixation and the comparative absence of artifacts. The action of the gold chloride is, however, no more certain in this method than in others.

Mallory's (:00) method for study of the central nervous system proved useful in the case of Amphioxus for the peripheral nervous system. Vom Rath's fluid (strong) was employed, but with indifferent results, except for the central nervous system. The methods of Golgi for impregnation with silver, and their various modifications, are all useful in obtaining impregnations of different parts of the nervthe anterior portion of the first myomere. Occasionally little of the main stem of nerve I is visible; in other cases it may be observed for almost its entire length, while in still other specimens its union with the neural tube, and even a portion of the tube itself, are exposed to view. The main stem usually lies close to the dorsal surface of the chorda.

Nerve II.— In 1841 Johannes Müller described the second nerve, enumerating it as the first (J. Müller **'44**, p. 95, 96). Quatrefages (**'45**) numbered the branches of nerve II as nerves II, III, IV, and V, and figured the terminal branches of nerve I in the rostrum as arising from nerve II. This conception was based upon his idea that the first was an *optic* nerve, a short stalk connecting brain and "eye spot." Schneider (**'79**) figures (Taf. 15, Fig. 1) two roots for nerve II of the right side, stating (p. 14) that this nerve usually possesses two roots upon one side, and upon the other divides immediately after its origin.

Dogiel (**: 02**) gives an extended and accurate account of the distribution of the branches of nerve II, as well as the variations which occur in the form of the nerve itself, and the territory which it innervates. He finds from one to three nerves in the region of the exit of nerve II, conditions not being symmetrical in this respect on the two sides of the body. He enumerates these irregularly occurring nerves as distinct nerves, without considering their relation to the myomeres. Although his figures do not present the muscle segments with clearness, they convey an inaccurate idea of the number and position of these segments. Dogiel describes several forms of variation from the usual course to the rostrum of nerves I, II, and III (as he enumerates them). In one case nerve II is poorly developed, and a branch of his nerve III innervates the ventral part of the rostrum (Dogiel's Fig. 6*b*, which illustrates this condition, shows the roots of nerves II and III in close proximity). In another case, of frequent occurrence, nerve II is strongly developed and only the posterior ventral part, if any, of the rostrum receives branches of nerve III. (In Dogiel's Fig. 6*c*, and 6*d*, illustrating this case, the nerve designated as III is the one commonly accepted as III.) Occasionally his nerve III (shown in his Fig. 7, which is not the nerve usually enumerated as III) ramifies exclusively in the skin of the dorsal fin, a ventral branch being absent. In such cases the skin of the posterior ventral portion of the rostrum is supplied by branches from his nerve IV. (From Dogiel's Fig. 7 this is clearly the commonly accepted nerve III.) In another form of variation (Dogiel's Fig. 8*a*, and 8*b*) his nerve III consists of two or three dorsal branches and a small ventral ramus, which does not reach the ventral border of the rostrum. According to his figures this nerve (III) makes its exit near the root of nerve II. In a few cases (illustrated by his Fig. 9) Dogiel observed directly posterior to the large root of nerve II two small nerves, which he designated as nerves III and IV. Neither of these possesses a ventral ramus which reaches to the ventral border of the rostrum; in such cases the skin of the rostrum is in part supplied by his nerve V. It is clear from his figure that the nerve numbered V is the one usually designated as III.

From the foregoing description it will be seen that Dogiel does not regard the anterior sensory nerves as occupying definite relations to the myomeres. Changes in the numbering of the anterior sensory nerves must of course affect the numbering of all the other sensory nerves. Thus one, two or three roots in the region of the exit of nerve II, if designated in accordance with Dogiel's view as separate nerves, would correspondingly change the numbering of all the succeeding dorsal nerves, which show such a constant relation to the muscle segments. Since the two sides are frequently not symmetrical with regard to the number of roots in the region of nerve II, one might have, according to Dogiel's system of enumeration, the correspondingly numbered nerves of the two sides belonging to myomeres *not* correspondingly numbered.

I have examined a large number of specimens, frequently with the aid of artificial light, and find that roots accessory to the main root of nerve II are often present, as is shown in my Figures 11 and 12 (Pl. 3). These two figures present the two sides of the same individual and illustrate the manner of exit of the roots of nerves II and III, and their relation to the myomeres. They also show the lack of symmetry in the roots of nerve II on the two sides of the body. Figures 6 and 7 (Pl. 2) show other conditions in the roots of nerve II. These do not differ in their form and position from the nerves described by Dogiel for this region. The main root of nerve II, and those roots that make their exit in close proximity to it, always lie anterior to the first myomere of the adult. Small roots may lie considerably posterior to the main root of nerve II and still occupy a position anterior to the first myomere. This is possible owing to the form of the muscle segment. Examination of a large number of individuals leaves in my mind no doubt of this condition. A distinct myomere intervenes between the root (or roots) of nerve II and that of the commonly accepted nerve III (Figs. 11 and 12). Moreover, the territories innervated by these nerves, while subject to some variation, are comparatively well defined. The dorsal nerves make their exit in definite relation to the myomeres, as is illustrated in Figure 6, and a logical enumeration of the nerves could not allow the association of the same myomere with different nerves in different individuals. Nerve III has a definite character and territory of distribution; its number should not depend upon the number of roots possessed by nerve II, nor on their being variously designated as nerves III, IV or V. It is not an unknown condition for a dorsal nerve in the more posterior parts of the body to have two distinct roots making their exit through the same myoseptum. The possible cranial character of the nerves anterior to the first myomere in the adult can scarcely authorize the enumeration of each separate root in the region of the place of exit of nerve II as a distinct nerve root; because the lack of symmetry on the two sides of the body, and the irregularity of the occurrence of these roots, would leave no basis for rational enumeration. The fact is that nerve II supplies a fairly definite territory, consisting usually of a large part of the rostrum and an adjoining portion of the dorsal fin.

I have not observed the small muscle segments which Dogiel figures (his Fig. *6a*, *6b*, *8b*) anterior to the origin of nerve II. The anterior boundary of the first muscle segment of the adult is often difficult to distinguish, and in my own observations strong artificial light was frequently employed to make this clear. Careful camera drawings were made of the dorsal portions of the anterior myomeres, as shown in Figures 6, 11 and 12. In specimens of both species the anterior projection of each muscle segment forms a much more acute angle than Dogiel's figures indicate. In this connection Hatschek's ('92) views regarding the first myomere may be mentioned. He states that in the larvae of Amphioxus the muscle fibrillae of the rostral process are well formed even out to the tip of the rostrum; but in the fully developed animal they become rudimentary, only a remnant persisting. In transverse sections of young Amphioxus (not larvae) treated with Mallory's differential stain, I have observed the cut ends of muscle fibers, lying in front of the anterior border of the first adult myomere. These fibers were short and few in number, but clearly differentiated by the stain from the surrounding connective tissue.

Nerve II was studied to a considerable extent in Branchiostoma caribaeum, and its distribution in the rostrum of this species is shown in Figures 1–5. As this species has been little illustrated, it is thought best to reproduce the conditions rather fully, for such figures form an interesting basis of comparison with Branchiostoma lanceolatum. No very essential differences, however, occur between the two species. Variations in the number and arrangement of the roots of nerve II are probably as frequent in one form as in the other. Hatschek (**'92**, Fig. 6) figures a branch of nerve II as passing to the deeper nerve plexus of the mouth. I have never observed such a branch, but am aware that variations occur. However, such a condition can scarcely be the typical one. Nerves I and II usually send no branches to deep lying structures.

No new observations concerning the olfactory pit were made in the present study, and this structure has not been included in the enumeration of the dorsal nerves.

With regard to the spinal or cerebral character of the more anterior dorsal nerves, and especially those having no ventral roots, there has been much variation of opinion. Owsjannikow (**'68**) and Stieda (**'73**, p. 48) designate the first two nerve pairs as cranial. Stieda, however, denies the existence of nerves of special sense. Langerhans (**'76**, p. 279) says that the first and second nerves are distinguished from the others by the possession of peripheral ganglion cells, as well as by their origin. Schneider (**'79**, p. 14) regards the first and second nerves, and the bulbus olfactorius as cranial, but considers it uncertain whether they differ in function from the remaining sensory nerves. Rohde (**'88**) calls the first five sensory nerve pairs cerebral. It does not fall within a discussion of the distribution and structure of the peripheral nerves, to treat of the question as to the cranial or spinal character of the more anterior dorsal nerves. The structure of the neural tube, and the functions of these nerves must enter largely into such a determination. If, as Hesse (**'98**) states, the whole spinal cord reacts to light stimulation, we may well make careful investigation of the physiological character of the more anterior nerves, before ascribing any special degree of cephalization to Amphioxus. Heymans et van der Stricht (**'98**, p. 68) say, in regard to this question, that the anterior extremity of the spinal cord of Amphioxus might perhaps be considered anatomically as a head (in its first stage of development), but that physiologically the head perhaps extends over the whole nerve axis, though especially developed in the segment corresponding to the buccal and branchial region.

B. Nerves of the Buccal Region.

Nerve III.— The third dorsal nerve usually sends a small branch, or branches, to the posterior part of the rostrum, but by far the greater part of its branches are confined to the buccal region. It may, therefore, be properly included with the nerves of this group.

Certain earlier authors, as Schneider ('79) and Rohon ('82), regarded the third pair of dorsal nerves as the first true sensory spinal nerves, this interpretation being based partly on the fact that the first pair of motor roots is associated with this pair of sensory roots. These authors enumerated the "spinal nerves" separately, designating the third sensory pair as the first spinal sensory roots. At present it seems safest to enumerate this pair of dorsal nerves as the third, without attempting to distinguish between so-called cranial and spinal nerves.

Heymans et van der Stricht ('98) have given a careful account of the distribution of the branches of this pair of nerves in Branchiostoma lanceolatum, and Dogiel (: 02) discusses nerve "III" in the same species at considerable length. As has been noted, however, Dogiel ascribes no definite territory of distribution to the third pair of nerves, its number depending upon the number of roots present near the place of exit of nerve III from the neural tube. This uncertainty makes comparison difficult and much involved. It may be stated, however, that in his discussion of the innervation of the border of the mouth Dogiel designates the commonly accepted third nerve as III, as is shown in his Figur 1.

I have studied this nerve in both species, chiefly upon specimens impregnated with methylene blue, although gold chloride and picrocarmine bring out excellently many details in the nerves of the oral hood. The finer branches of the nerves of the buccal region are usually impregnated in from 15 to 30 minutes in the methylene-blue mixture previously described, while the main trunks of these nerves require a longer immersion. A rather long subsequent exposure to air (20 to 30 minutes) gave peculiarly brilliant results for the finer branches. Fixation with ammonium molybdate and osmic acid, after Dogiel's (: 02) method, is well adapted to this region. The thin tissues fix rapidly, and may be dehydrated quickly without loss of impregnation. It is advisable before fixation to detach the anterior portion of the animal, cutting a little behind the velum. By dissecting along the mid-dorsal line, a flat, fairly thin preparation may be obtained for mounting in balsam. Thick pieces of tissue fixed in ammonium molybdate are usually unsatisfactory for sections or other methods of study.

In the two species studied surprisingly little difference was found in the distribution of the branches of the third pair of nerves. Variations occur as frequently in Branchiostoma caribaeum as in B. lanceolatum, and are similar in character. In both species nerve III usually possesses a dorsal and a ventral ramus, although in rare cases either may be absent, with the possible exception of the ventral ramus of the left side. I was unable to find any specimens in which the latter branch was entirely lacking. Occasionally the root subdivides at or near its place of exit from the neural tube, and either ramus is represented by two or more branches. The primary division in nerve III usually occurs near the neural tube, but considerable variation exists in this regard. Different forms of division in the two species are illustrated in Figures 6, 7, 11 and 12. In these figures the comparative uniformity in the place of exit of the nerve through the myoseptum may be noted.

The curious post embryonic history of the buccal region brings about the well known asymmetry in certain of the buccal nerve pairs. The dorsal ramus of the third nerve of either side usually innervates the neighboring portion of the dorsal fin, and a small area overlying the adjoining trunk muscles. A small branch of the ventral ramus of nerve III on either side of the body supplies the posterior ventral portion of the rostrum. Twigs from this branch may anastomose with branchlets of nerve II. In its course over the side muscles, this more anterior ventral branch of nerve III gives off numerous subdividing side branches. The main branch of the ventral ramus of either side takes a course ventrad and noticeably anteriad over the side muscles, and also gives off a number of side branches destined for the skin of this region. In this part of its course, or at the ventral border of the trunk muscles, this main branch of the ventral ramus of the third nerve, on either side of the body, gives off a branch (or branches) which passes to the anterior portion of the border of the mouth of the corresponding side and breaks up into the outer mouth plexus of this region. This branch usually leaves the more ventral side of the main branch, but may vary considerably in size and position. The main ventral branch of left nerve III is usually of greater size than the corresponding branch of the right side, and supplies a larger territory. This main branch divides at or near the ventral border of the trunk muscles of the left side into two branches, one of which passes beneath the ventral border of these muscles and emerges on the right side of the body, where it is continued across the oral hood to form the anterior portion of the inner mouth plexus of this side. The other branch traverses the oral hood on the left side, and breaks up into the left anterior portion of the inner mouth plexus. Thus the most anterior portion of this inner plexus on either side of the hood, is supplied by branches from left nerve III, while the outer mouth plexus of the same region is formed symmetrically from branches of nerve III, one the right nerve, the other the left. Heymans et van der Stricht ('98) also note this condition in Branchiostoma lanceolatum. Dogiel (:02) describes in detail the structure of the plexuses of the mouth border. These plexuses will be briefly considered later in this paper. I found no branches of left nerve II joining the inner mouth plexus, as described by Hatschek; on the contrary, the specimens observed indicate that the second nerve pair is not concerned in the innervation of the deep-lying structures of the mouth border.

Interesting variations occur in the distribution of the branches of nerve III in both species. In one case (B. lanceolatum) a branch of right nerve III anastomosed with the branch of left nerve III on the right side, thus actually joining the inner mouth plexus. Ordinarily the ramifications of left nerve III in the inner mouth plexus on either side join each other near the median line, but in one specimen (B. caribaeum) no anastomosis occurred, thus presenting the curious condition of an incomplete "nerve ring" in the inner mouth plexus.

Nerve IV.— This nerve usually impregnates with methylene blue coincidentally with nerve III, but the portion extending over the trunk muscles often shows a better impregnation after longer immersion. Bilateral asymmetry exists in the fourth nerve pair, as in the third pair, and many variations occur in its manner of division, and the distribution of its branches. As shown in Plate 2, Figure 6 (B. caribaeum), nerve IV arches slightly more than nerve III immediately after its exit from the neural tube. Near its place of exit, this nerve divides into a dorsal and a ventral ramus. More than one dorsal ramus may be present, and the ventral ramus may subdivide near its origin; but I have never noted the entire absence of the ventral ramus, or its equivalent, on either side of the body. Occasionally the dorsal ramus of a sensory nerve is absent, or represented by a twig of practically no importance. In such cases its territory is supplied by branches of an unusually well developed dorsal ramus of one of the two adjoining nerves. The place of division of nerve IV into a dorsal and a ventral ramus may be readily noted in Figure 6. This place of division is often concealed by the muscles, but frequently may be ascertained by the aid of strong light, especially in methylene-blue preparations preserved in glycerine and ammonium picrate. Such preparations often become very transparent. Heymans et van der Stricht ('98) say that the bifurcation of the dorsal roots into dorsal and ventral rami is always made before exit from the myoseptum, except in the case of the last caudal root. According to my observations, the place of division in the first 6–8 anterior nerves may occur at, or slightly after, the exit of the nerve from the myoseptum, although it frequently occurs nearer the neural tube.

The stem of the dorsal ramus of nerve IV is usually deflected more or less anteriorly (Figs. 6 and 7, Pl. **2**). This ramus divides repeatedly, distributing its branchlets over the surface of the neighboring trunk muscles, and in that portion of the dorsal fin lying approximately dorsal to the nerve root. The ventral ramus of nerve IV is usually of greater size than the corresponding ramus of nerve III, especially upon the left side. This ramus of nerve IV takes a general ventrad course over the trunk muscles; but in its course over these muscles it forms an arch the convexity of which is directed anteriorly. This arch is frequently more prominent on the left side, and a like one may also be noted in the ventral ramus of nerve V (Pl. **2**, Fig. 7). Dogiel (: **02**) states that an arch is also formed in the course of the ventral ramus of nerve III, and occasionally in nerve VI. I, however, have observed a *marked* arch only in the course of nerves IV and V. On the left side this arch is accentuated by the fact that the branch of this ramus which innervates internal substructures bends toward the interior of the body. The ventral ramus of nerve IV gives off numerous subdividing branches in its course over the side muscles. On the right side it divides into two or more descending branches, which innervate a portion of the outer wall of the oral hood, and finally break up into a part of the outer mouth plexus. These descending branches may be formed either in the course of the ventral ramus over the trunk muscles, or at the ventral border of these muscles, or even in the oral hood. The branches which cross the oral hood usually give off only small side branchlets. The ventral ramus of left nerve IV innervates a much larger territory, and shows considerable variation in the number of its branches, and their manner of distribution. This ramus usually divides into its main branches near the ventral border of the side muscles, but often varies in this regard. A branch, or branches, to the outer plexus of the mouth may be given off from the main branch at any point in its course over the trunk muscles (Fig. 7). Other branches to this plexus may be given off at or near the place of main division mentioned above.

The remaining branches formed at this place of division are destined for less superficial structures. Of these, one or more take a course ventrad across the oral hood and form that part of the inner plexus of the mouth lying posterior to the portion formed by branches of left nerve III. The branches of left nerve IV which aid in the formation nection between nerves V and VI is illustrated in Figure 7. As has been stated, a branch of left nerve IV sometimes anastomoses with left nerve V in this region; left nerves VI and VII often show some form of connection with it in the neighborhood of the ventral border of the trunk muscles. Thus, with the slight connection sometimes occurring between nerves II and III, a basis is formed for the band-like nerve described by Hatschek ('92) as connecting nerves II–VII in this region. Hatschek's Figure 6 must be somewhat diagrammatic, however, since this condition is found only on the left side, and even here these connecting nerves are not constant in occurrence or size. In nearly all cases left nerve V sends one or more branches to the velum. These branches usually leave the ventral ramus near the ventral border of the side muscles (Figs. 7, 8), and enter the velum on the left side of the body. At least one of these branches forms an anastomosis with a branch or branches of left nerve VI, and frequently a similar anastomosis is formed with a branch or branches of left nerve VII. These anastomoses vary greatly in character; it is often difficult to determine the share of each nerve in the innervation of the velum. This is especially true when, as sometimes happens, branches are given off to the inner mouth plexus ventral to the anastomosis between branches of left nerves V and VI (Fig. 7).

Nerve VI.— This nerve requires only a brief description. A form of division of the trunk of this nerve in B. caribaeum may be noted in Figure 6 (Pl. 2). The dorsal rami of this nerve pair are similar to those of the fifth pair of nerves; their main branches are illustrated in Figures 6 and 7. The ventral ramus of the sixth nerve, both the right and the left, is usually smaller than the corresponding ramus of nerve V, but the cutaneous branches given off in its course over the trunk muscles correspond to those leaving the similar portion of nerve V. In both species the remaining branches of right nerve VI are distributed in the oral hood and external portion of the mouth border of the right side. The ventral ramus of right nerve VI divides near the ventral border of the trunk muscles into two or more slender branches, which take a general course ventrad across the oral hood, and break up into a portion of the outer mouth plexus. The corresponding ramus of left nerve VI sends a similar branch, or branches, to the outer plexus of the mouth border on the left side of the body. Left nerve VI frequently sends a branch, or branches, to the inner plexus on the left side of the mouth, but this is not uniformly the case. In several specimens examined branches of left nerves III, IV, and V formed the entire left portion of the inner mouth plexus. Heymans et van der Stricht ('98, p. 36) state that the plexus of Fusari is formed from branches of left dorsal nerves III–VI, and perhaps VII. As has been stated, a branch of left nerve V usually anastomoses with a branch, or branches, of left nerve VI. From this anastomosis a branch passes to the velum, frequently uniting in its course with a branch of left nerve VII (Fig. 7). This branch to the velum may divide in various ways before entering the latter structure. Its branches, however, enter the velum on the left side of the body.

Nerve VII.— Figure 6 (Pl. **2**) shows this nerve leaving the neural tube slightly nearer its dorsal surface than nerve VI. The dorsal ramus of nerve VII on either side of the body is similar to that of nerve VI, except for a slight increase in size, due to the larger territory lying dorsal to the seventh nerve root. The ventral ramus of nerve VII on either side of the body does not differ from that of nerve VI in the region of the trunk muscles. Right nerve VII sends one or more branches to the posterior portion of the outer mouth plexus. These branches give off small side branches in their course across the oral hood. Nerve VII of the right side occupies a position considerably posterior to that of left nerve VII, and its ventral ramus frequently lies exterior to the velum. In such cases right nerve VII is usually the most posterior nerve of the right side sending branches to the outer plexus of the mouth border. In nearly all the specimens examined the branches of left nerve VII were found to be exclusively cutaneous in their distribution. This nerve sends branches to the outer mouth plexus of the left side, similar to the corresponding branches of right nerve VII. I have been unable to find a branch, or branches, of left nerve VII in either species directly connected with the inner plexus of the mouth border. As has been noted, Heymans et van der Stricht ('98) state that left nerve VII is perhaps concerned in the formation of the plexus of Fusari. This may be the case in rare instances, but is certainly not the usual condition. These authors also state that left nerve VII contributes toward the innervation of the velum. Dogiel (: 02) also says that nerve VII not infrequently takes part in the innervation of the velum. The specimens examined by me revealed no large branches of left nerve VII passing directly to the velum. In a few cases a small branch, or branches, of this nerve anastomosed with branches of left nerve V or VI (Figure 7); therefore fibers from branch VII may take this course to the velum; but it is difficult to determine the course of nerve fibers after such an anastomosis.

Nerve VIII.— This nerve will be briefly considered here, although its branches, for the most part, supply the branchial region. It may be noted in Figure 6 that this nerve leaves the neural tube very near its dorsal surface, and arches noticeably immediately after its exit. The dorsal ramus of nerve VIII on either side of the body, and the branches of this nerve pair in the region of the trunk muscles, are so similar to the corresponding branches of the two next anterior nerves, already described, that a separate description is unnecessary. I have been unable to find a branch of the ventral ramus of right nerve VIII which unquestionably joins the outer plexus of the mouth border. However, since branches of this nerve sometimes lie considerably anterior to the velum, it seems probable that they may occasionally contribute toward the formation of that plexus. Left nerve VIII frequently sends a small branch to this plexus (Fig. 7), but such a branch is as frequently lacking. None of the specimens examined showed a branch of left nerve VIII passing to the velum, as described by Dogiel (:**02**).

The variation in the territory innervated by the individual anterior nerves is of considerable interest, since the branches of a nerve usually supplying a certain area, may be supplanted by those of another nerve which leaves the neural tube at some distance from the place of exit of the nerve ordinarily innervating the given territory. It appears from this that the same nerve does not always carry fibers to precisely the same area. The plexuses and communicating nerve bands of this region may have a bearing on this point, and lead to doubt as to which nerves actually supply certain territories. The relation of the nerve components of the neural tube to each other in this region must also be of interest. It is certainly a fact worthy of consideration that a great nerve, like nerve IV, leaving the neural tube at a definite place, may or may not take a prominent part in the innervation of the velum.

The plexuses of the mouth border have been frequently described, especially by Fusari ('**89**) and Dogiel (:**02**), and will not be taken up in detail in this paper. Besides the inner and outer plexuses of this region, Dogiel describes a "Zwischengeflecht," lying between them and taking its origin chiefly from the outer plexus. The nerve branches taking part in the formation of the plexuses of the mouth border in the specimens of both species observed by me have been already enumerated. Figure 18 (Pl. 4) illustrates a portion of the outer plexus in Branchiostoma caribaeum. This plexus does not appear to be essentially different from the corresponding plexus in B. lanceolatum. The inner plexus is also apparently similar in the two species.

C. Nerves of the Velum.

The nerves of this structure have been so little described and figured that they will be considered under a separate heading in this paper. Rathke, as early as 1841, mentions the velum, giving the number of tentacles as sixteen. Rolph ('76) notes that the velum carries about ten cirri. Rohon ('82), van Wijhe ('93), and Hatschek ('92) mention nerve branches passing to the velum; but the most detailed description of the innervation of this structure is found in the work of Heymans et van der Stricht ('98). These authors state that the voluminous circular nerve of the velum is formed from branches of left dorsal nerves IV, V, VI and VII, and appears to occupy about the middle part of the sphincter muscle, at the interior of which it gives off large branches. With regard to nerves passing to the velar tentacles, these authors state (p. 36): "nous avons également observé à l'intérieur des tentacules des fibres nerveuses que nous avons pu poursuivre jusque entre des cellules épithéliales." Contrary to van Wijhe, they found in their preparations no impregnation of sense cells in the velum. As mentioned in the discussion of nerve IV, Heymans et van der Stricht describe a posterior branch of left dorsal nerve IV which passes under the body of Hatschek, and reaches the right portion of the velum. These authors illustrate (Pl. VI, Fig. 22) the large nerve branches passing to the velum, and also its circular nerve. Dogiel (:02) finds branches from left nerves IV, V, VI, VII, and even VIII and IX, taking part in the innervation of the velum. The branches of nerves IV and V are usually, however, most important. Dogiel's enumeration of the more anterior nerves must be considered in connection with this statement. This author shows (Taf. 20–21, Fig. 13) a portion of the plexus of the circular muscle of the velum. In this figure branches of left nerves V, VI and VIII form the velar plexus. In his Figur 1, branches from left nerves IV, V, VI and VIII pass to the velum. The branches of nerves VIII and IX which he finds occasionally passing to the velum are fine branchlets of the rami viscerali of these nerves.

My personal observations on the innervation of the velum were made largely from dissected specimens of Branchiostoma lanceolatum and B. caribaeum impregnated by the intra-vitam method with methylene blue. Most of these specimens were fixed in ammonium picrate, and preserved in a mixture of the ammonium picrate and glycerine. A few specimens were fixed in ammonium molybdate, dissected and mounted in balsam. In either case, a few drops of osmic acid added to the fixing fluid appeared to aid greatly in preserving the impregnation. It was usually most convenient to use only the anterior portion of the body in studying the velum, severing the body a short distance behind that structure. This anterior portion was prepared for study under a dissecting microscope by cutting with fine scissors along the longitudinal axis, usually on the right side, as close as possible to the dorsal fin. Certain specimens were cut in different regions, so as to make it possible to determine the course of the nerve branches on both sides of the body. These preparations were arranged on a slide in the preserving mixture, with the aid of camel's hair brushes. The velar tentacles were arranged in the position showing the nerves to best advantage. Thin supports beneath the cover-glass were usually necessary to prevent injury. Strong light was found useful in following the deep-lying nerve branches, but the velar plexus, when impregnated, is usually visible with ordinary light. The velar nerves require a long immersion in the methylene-blue mixture (1½ to 2½ hours), with a subsequent exposure to air of about one hour. Unless osmic acid is added to the fixing fluid, the epithelial covering of the velum sloughs off to a large extent.

Although considerable variation exists in the innervation of the velum, Figure 8 (Pl. 2) presents a fairly typical picture of the branches of the dorsal nerves which pass to this structure, and the distribution of the nerves in the velum itself. Figure 9 shows the nerves of a large and a small velar tentacle in greater detail. These figures present the first published views of the entire plexus of the velar muscle, and of the nerves of the velar tentacles. These figures do not show the epithelial covering of the velum, except for occasional cells apparently remaining attached to nerve branches.

Figure 8 may be taken as a basis for the description. In this specimen branches of the ventral rami of left nerves V and VI supply the velum. A somewhat complicated alternation of anastomosis and division takes place between these branches as they pass to the velum. A large branch of left nerve V takes a course along the ventral muscle border to the ventral ramus of left nerve VI, and anastomoses with the latter. Two branches destined for the velum are given off near this place of union. The larger (α) takes a backward course along the ventral border of the trunk muscles for a short distance, and then bends around the muscle border to the inner surface of the side muscles, where it is continued posteriorly with considerable deflection dorsalward. As it bends toward the interior, this large branch gives off a much smaller one (γ), which passes posteriad and ventrad, dividing after a short distance into two branches, which reach the velum a short distance ventral to the muscle border. These two latter branches unite in the velar muscle, and the band-like nerve thus formed may be followed in almost the entire circumference of the ring muscle, although its size varies considerably in different portions.

The smaller branch (β), leaving the place of anastomosis between the branches of nerves V and VI, takes a general course backward over the inner surface of the trunk muscles, running for a short distance dorsal to the larger branch. Near the velum these two nerve branches (α, β) unite, forming a short, thick trunk. This trunk divides into two short, diverging branches of equal size. The more dorsal branch (b') enters the velum about the width of two myomeres dorsal to the ventral muscle border of the left side, and is continued dorsad in the circular muscle of the velum as a slightly irregular, thick band. This band curves over to the right side of the velum, and breaks up into the velar plexus. The more ventral (δ) of these short, diverging branches continues ventrad in the ring muscle of the velum, losing its band-like character at the base of the fifth tentacle ventral to the border of the trunk muscles. These band-like nerves lie chiefly near the bases of the skeletal portions of the velar tentacles, but do not form a continuous "band nerve." The velar plexus lies, for the most part, between these thick, flat nerves and the free portions of the tentacles. A comparatively large branch passes to each tentacle obliquely from one of the band-like nerves; this oblique deflection is usually ventralward, but may vary. A single large tentacle in the mid-ventral region receives two main branches, one from the right side, and one from the left. The velar plexus is completed by numerous small branches, which anastomose to form an irregular, loose-meshed network between the band nerves of the circular muscle and their large branches which supply the tentacles. Toward the more external border of this plexus many apparently free-ending small nerve branches are visible. These may terminate in some form in the velar epithelium, or may possibly join a finer unimpregnated plexus.

The nerves of the velar plexus generally exhibit a flattened, granular appearance when impregnated with methylene blue, and their outlines do not present the clear-cut aspect noticeable in cutaneous branchlets of dorsal nerves. Their structure calls to mind that of the nerve branches forming the inner plexus of the mouth border, and the inner abdominal plexus. Apparent oval nuclei, or nucleated cells, may be frequently observed in the course of the finer branches of the velar plexus (Pl. 2, Fig. 9).

The tentacles of the velum are usually alternately large and small, but this arrangement does not obtain with absolute regularity. Their number does not appear to be constant, but is in the neighborhood of fifteen. The innervation of the tentacles presents a beautiful appearance when impregnated with methylene blue. Figures 8 and 9 illustrate these nerves with different degrees of detail. From these figures it may be observed that each large nerve passing to a tentacle breaks up suddenly, at the base of its tentacle, into a great number of small branches, which divide, anastomose and interlace throughout the entire free portion of the tentacle. Such meshes as are formed are usually much elongated parallel to the long axis of the tentacle. These nerves of the velar tentacles are also granular in appearance, and occasional small nuclei or cells occur in the course of the nerve threads. Free nerve ends may be noted, but these must always be considered in connection with the fact that in methylene-blue preparations little epithelium favorable for studying the general innervation of the velar tentacles persists. In some instances small, darkly-staining thickenings of the nerve ends may be seen projecting beyond the border of the tentacles, or exterior to the network of nerves (Fig. 9, *c*). These may represent some form of nerve ending in the epithelium of the velar tentacles. The elaborate innervation of the tentacles suggests that many nerve endings may exist in them.

The variation mentioned in the nerves supplying the velum has no doubt led to the apparently conflicting statements with regard to the numbers of these nerves. In the specimens observed, only branches of nerves of the left side of the body passed to the velum, and these usually enter the velar muscle on the left side. A branch of left nerve IV has been described which entered the velum slightly to the right of a mid-dorsal line, but this case appeared to be exceptional. Nerve IV may send a branch directly to the velum, or a branch may anastomose with that of nerve V on its way to the velum. In Figure 7 branches of nerves VI and VII anastomose with the branch of nerve V passing to the velum. It is difficult to explain just what these anastomoses mean. Nerve IV sometimes sends a large branch to the velum, nerves V and VI usually send branches of considerable size, but nerve VII seldom contributes largely toward the innervation of the velum. The branch of left nerve IV indicated at IV*a* in Figure 8 appears to be associated with the nerves of the velum, but is actually entirely free from the velar muscle. I have frequently observed a branch of left nerve IV ending in this manner in this region. Variation no doubt occurs in this nerve branch, and it may therefore correspond to the branch of nerve V described by Hatschek ('**92**, Figur 6, *N. S.*) as passing to the groove of Hatschek. Heymans et van der Stricht ('**98**) state that the N. recurrens of Hatschek ends at the velum, and has nothing in common with the branchial plexus. I have never observed a large branch from the buccal nerves reaching the branchial basket.

D. *Nerves of the Branchial Region.*

In this group are included the dorsal nerves lying between the velum and the region anterior to the atriopore. The term "branchial" is therefore unsatisfactory in some respects, but is perhaps the one most available for use in a description of the nerves. In B. lanceolatum this region is supplied by nerves VIII to XL, or XLI, inclusive, while in B. caribaeum it seems probable that nerves XXXVIII and XXXIX innervate the region of the atriopore. This indicates that the difference in the number of myotomes in the two species occurs, in part, in the branchial region. Dogiel (:**02**) states that in B. lanceolatum nerve XLII is the most posterior nerve supplying the region of the atriopore. He describes the fine-meshed plexus ramifying about this opening, and finds that branches of nerves XXXVIII–XLII may take part in its formation. Variations certainly occur in these nerves, as in the case of other dorsal nerves.

The nerves of this region impregnate particularly well with methylene blue, but require a longer immersion than those of the anterior part of the body, especially in the case of the visceral branches. Strong light was very useful in following the finer nerve branches in the thicker portions of the body. Various branches of these dorsal nerves have been repeatedly described; the accounts, which are somewhat fragmentary, will be taken up in connection with the description of my personal observations.

The dorsal nerves of this region differ little from each other in their main characteristics, and in certain respects resemble those of the buccal region. It may be noted that, as seen in Plate **2**, Figure 6, the arch formed by each dorsal nerve as it leaves the neural tube gradually increases in height toward the middle region of the body. Here the arch remains practically constant, but toward the posterior region it again diminishes in height, and at the extreme posterior end, as will be noted later, no arch is present. In the anterior portion of the branchial region the place of division of the dorsal nerves into dorsal and ventral rami is usually at, or just before, the exit of each nerve from the myoseptum (Fig. 6). In the remainder of this region the place of division lies deeper, due, in the main, to the greater thickness of the trunk muscles; for the majority of the nerves of this group divide at a comparatively uniform distance from the neural tube. Much variation exists with regard to the place of exit of the dorsal and ventral rami through the myoseptum (Pl. **6**, Fig. 34; Pl. **7**, Figs. 37, 39). Johnston (:**05**) states that "the typical place of division is about half way between the cord and the dermis," rather than close beneath the dermis as described by Hatschek. Johnston finds much variation in the place of division, and frequent cases of separate emergence of the dorsal and ventral rami.

The dorsal rami of the nerves of this group resemble those of the more anterior region. They are largest in the thickest portion of the body, and possess a great number of branches, and, like the anterior dorsal rami, innervate the cutaneous area over the neighboring trunk muscles, and the adjoining portions of the dorsal fin. These dorsal rami frequently emerge from the myoseptum not as single stems, but in several branches, division having taken place beneath the dermis (Figs. 34, 39).

The ventral rami of these nerves are usually large, but may vary, one or two rami being increased in size at the expense of an adjoining ramus (Fig. 39). A ventral ramus may even be entirely absent. Both dorsal and ventral rami give off a number of repeatedly dividing branches in their course over the trunk muscles. In many methylene-blue preparations which retain little epithelium, the finer branches appear to end in innumerable delicate branchlets, the distal ends of which are often noticeably directed outward. Frequently these slender branchlets are continued into fine threads, which anastomose with one another and with similar threads proceeding from adjoining nerves, forming a delicate, irregularly meshed plexus. Such plexuses are not limited to this region, but are also present in connection with nerves posterior to the atriopore. These plexuses have been so little observed and figured that a detailed account will be given of their distribution and character in connection with the discussion of sensory nerve endings. The ventral rami of the dorsal nerves of the branchial region (with a few possible exceptions) divide near the ventral border of the side muscles, to form three main branches (see sketch, Pl. **4**, Fig. 15a). This division is accomplished in various ways, and each resulting branch may be represented by one or more twigs. The most exterior branches formed by this division are the *rami cutanei ventrales*, noted by early authors, and well described by Fusari ('**89**), Heymans et van der Stricht ('98) and Dogiel (: 02). These branches ramify over the metapleural fold and ventral wall of the atrium, forming a cutaneous network, the meshes of which are generally elongated longitudinally. Nerve VIII is usually the most anterior nerve sharing in the formation of this plexus. Fusari ('89) describes nerve cells and ganglionic knots in connection with this network. It is of interest to note that these cutaneous abdominal branches may leave the ventral ramus at or near the ventral muscle border, or the ventral ramus may divide much earlier in its course, sending one or more twigs from the branches thus formed to the superficial abdominal region (Figs. 37, 39). In Figure 39 the absence of a ramus cutaneus ventralis in connection with nerve XXXVI is to be noted.

The remaining two divisions of the ventral ramus are frequently formed from one main short stem; this may bend toward the interior beneath the ventral border of the trunk muscles, or may penetrate a myoseptum at some distance (the width of one, two or even three myomeres) dorsal to this ventral muscle border. These two branches may, however, leave the ventral ramus independently, instead of by a common stem. On the inner surface of the trunk muscles the short stem mentioned above divides into two main branches. One of these, the ramus visceralis ascendens, ascends over the inner surface of the trunk muscles, while the other, the ramus visceralis descendens, descends to the inner surface of the transverse muscles. Nerves VIII or IX, and the succeeding dorsal nerves of this region usually possess a ramus visceralis descendens, but I was unable to find a ramus visceralis ascendens anterior to that of nerve XII. The most anterior ascending visceral ramus is small, but the succeeding one is larger, showing the characteristic fan-like branching. It is entirely possible that branches pass to the most anterior branchial bars from the descending visceral ramus, or the inner abdominal plexus. In this connection it may be noted that the fan-like branches of the most anterior ascending visceral rami observed, were directed obliquely forward. The diagrammatic sketch, Figure 15a (Pl. 4), made from several transverse sections, illustrates a fairly typical form of division of the ventral ramus. This shows a branch of a ventral ramus penetrating a myoseptum the width of one myomere above the ventral muscle border. In these sections, stained with Mallory's differential stain, the connective tissue may be readily discerned on either side of the penetrating branch. Upon its exit on the inner surface of the side muscles, the ascending visceral branch takes a course dorsad over the connective tissue covering of the muscles.

This branch runs for some distance inside a gonadial pouch. The descending branch passes downward over the inner surface of the most ventral myomere to the inner surface of the transverse muscles. Here it appears to lie between layers of connective tissue. Both the ascending and descending visceral rami show a faint bluish tinge in sections stained with Mallory's differential stain, due, perhaps, to the presence of a thin connective-tissue sheath.

The distribution of the branches of the *rami viscerales descendentes*, forming the inner abdominal plexus, has been well described by Fusari ('89), Heymans et van der Stricht ('98), and Dogiel (: 02). Fusari and Dogiel note a network of extremely fine threads connected with the coarser meshes of the inner abdominal plexus. Dogiel describes these threads as somewhat varicose. He also finds other fine branchlets given off from the coarser plexus, which run to the abdominal muscles, and break up into a great number of repeatedly dividing finer threadlets that possess small varicose thickenings. Dogiel is of the opinion that these threadlets weave about the transverse muscles in an exceptionally thick plexus, analogous to that which he has described for the "ring-muscle" of the mouth. He does not find the fibers described by Heymens et van der Stricht as passing directly from the coarser plexus to penetrate between the lamellae of the transverse muscle, where they terminate in swellings analogous to the endings of nerve fibers in smooth muscle. Dogiel notes small three-cornered nuclei in the angles formed by branchlets of the coarser abdominal plexus. Fusari finds no ganglion cells in connection with this plexus, but notes small nuclei at the knotted places of anastomosis. He designates the nerves of the inner abdominal plexus as sympathetic.

The finer plexus described by Fusari and Dogiel is clearly visible in certain of my methylene-blue preparations. In these preparations the threads of this finer plexus are less varicose than those figured by Dogiel (: 02, Fig. 14, 15). However, at rather infrequent intervals swellings resembling small nuclei may be seen. Many of these finer threads cross the interior faces of the coarser meshes, indicating that they lie closer to the epithelium covering the inner surface of the transverse muscles than does the main plexus. A few fine threads, however, cross the external surfaces of the larger meshes. The meshes of the finer plexus are generally elongated parallel to the long axis of the animal. A secondary plexus is also present in that portion of the abdominal region which lies between the transverse muscles of either side of the body, but its meshes do not appear to be as close here as in other regions. In transverse sections stained with Mallory's differential stain, portions of the larger plexus may be seen lying on the inner surface of the transverse muscles, in connective tissue. Since many of the threads of the finer plexus are nearer the surface of the atrial cavity than those of the coarser plexus, they must be closely associated with the bases of the cells lining that cavity. In portions of my preparations the finer plexus is extremely complicated, and the meshes are not decidedly elongated. The meshes of the main inner abdominal plexus sometimes appear knotted in methylene-blue preparations, even though the knots are not differentiated in any way; but apparent nuclei are frequently present in the course of the nerve threads and at places of junction. Certain large meshes of the inner abdominal plexus send branches to the gonadial pouches (Pl. **3**, Fig. 13, *i.*), although the main supply for these structures is derived from the ramus visceralis ascendens. In Golgi preparations of Branchiostoma caribaeum, nerve threads were found scattered in the transverse muscles. It was impossible to determine their origin, but closely associated threads evidently did not all lie in the same focus. The greater number of these threads are arranged more or less nearly parallel to the long axis of the animal (Pl. **5**, Fig. 23).

Impregnation with methylene blue does not always produce the same results in the structural appearance of these visceral nerves. In some preparations the threads of the inner abdominal plexus appear granular, while in others distinct fibers can be traced. But notwithstanding these variations, the visceral nerves can always be distinguished from the cutaneous nerves by their rougher outlines.

Rami viscerales ascendentes.— Johannes Müller (**'41**, **'44**, p. 96), and Leuckart und Pagenstecher (**'58**) observed nerves passing to the branchial basket. Schneider (**'79**, p. 15) observed and figured sensory nerve branches penetrating to the interior at the ventral border of the side muscles. These branches ramify as they ascend on the surface of the abdominal cavity. He ascribes the function of the *vagus* to these nerves. Rohon (**'82**, p. 24) agrees with this interpretation. He also describes in some detail, the distribution of nerves in the ligamentum denticulatum, and in the branchiae. Fusari (**'89**, p. 130) describes the branchial nerve branch as ascending obliquely upward and forward after penetrating to the interior, finally joining the branchial apparatus by way of the ligamentum denticulatum. He states that only the more anterior nerves send branches to the branchiae and that the nerves of the tail also lack the "sympathetic" branch. It will be remembered that Fusari designates the nerves of the inner abdominal plexus as sympathetic. According to Rohon, the branchial nerves end in tufts. Fusari (p. 133) differs from this, and states that they spread out in a membrane that covers and supports the branchial apparatus on its external surface. On this membrane the different nerve branches form a net of irregular meshes, which Fusari calls the nerve net of the branchiae (his Taf. VIII, Fig. 2). This figure probably illustrates a plexus formed by ascending visceral branches before they reach the branchial basket (compare Pl. **4**, Figs. 15, 16). Fusari probably saw a nerve net distributed over the gonadic pouches. Heymans et van der Stricht (**'98**, p. 41–44) describe in detail the branches of the rami viscerales ascendentes, and figure nerves in the ligamentum denticulatum, a small number of fibers on the branchial bars, and fibers on the cross bars, joining those running lengthwise of the primary and secondary bars (their Fig. 30). These authors also figure a plexus distributed along the median ventral portion of the branchial basket, and show a portion of a plexus covering the gonads. They never found nerve fibers in the interior of the branchiae. They note a plexus in the ligamentum denticulatum, parts of which resemble a longitudinal nerve trunk; but in their opinion an actual large longitudinal nerve does not usually exist, except as formed indistinctly by the anastomosis of different branches of the ascending visceral nerves. These authors are therefore inclined to deny the existence of a distinct vagus nerve. Other branches of the rami viscerales ascendentes were observed, which these writers (Pl. VIII, Fig. 26) regard as perhaps innervating the blood-vessels, digestive tube, and the parietal serous surface of the branchial cavity. Dogiel (**:02**) presents figures of Golgi preparations showing numerous fibers on the primary and secondary branchial bars, and on the cross bars; also nerve cells of spindle-like or angular shape, previously undescribed, possessing from three to six processes, which gradually divide into a great number of twigs. Dogiel finds such cells in all parts of the branchial basket, and regards them as analagous to the sympathetic cells in the intestinal plexus of the "Neunauge" (river-lamprey). He also finds ascending visceral branches posterior to the branchial basket, which he is certain supply the intestinal canal (Fig. 17*a*, 17*b*, 18*a*, 18*b*, 19*a*, and 19*b*).

My own observations on the ascending visceral nerves and their branches were made chiefly from specimens of B. lanceolatum, only a few individuals of B. caribaeum proving favorable for study of these nerves. Dissected specimens previously impregnated with methylene blue were particularly useful in following the ramifications of the rami viscerales ascendentes. A study of these nerve branches is attended with difficulties, since it is necessary to lift the pharynx wall to disclose the nerves on the internal surface of the side muscles, and in the ligamentum denticulatum, and this proceedure is more than likely to break the slender nerve threads passing to the ligamentum denticulatum and the branchial basket. The accompanying series of figures (Pl. 2, Fig. 10; Pl. 3, Figs. 13, 14; Pl. 4, Figs. 15–21, Pl. 5, Fig. 30) aims to illustrate the distribution of numerous branches of the rami viscerales ascendentes with as much clearness as these technical difficulties permit.

As has been noted, a visceral branch may bend around the ventral border of the side muscles, or penetrate a myoseptum further dorsalward. A dorsal nerve may possess two or more visceral branches, instead of one. Figures 13 and 15 (Pls. 3 and 4) illustrate a condition of frequent occurrence, in which a branch of the ventral ramus penetrates to the interior through the myoseptum just dorsal to the most ventral myomere. In either case, division into a descending and an ascending visceral ramus takes place immediately after reaching the internal surface of the side muscles, the ascending branch running dorsalward, and the descending branch taking a general ventrad direction. In Figure 15 the descending ramus is small, but in Figure 13 its size is equal to that of the ascending ramus. Such variations in size are frequent, and when a branch is unusually small, compensation may ordinarily be noted in an adjoining nerve. The ramus visceralis ascendens gives off branches from either side as far as a main stem can be observed, but the origin of the more ventral of these branches is usually obscured by a gonadic pouch. When one of these pouches is empty (Fig. 13), the nerves which innervate its entire surface with an elaborate network may be readily seen arising either from the ascending visceral ramus, or its first branches, or from branches of the descending visceral ramus, or, finally, from meshes of the inner abdominal plexus. These nerve branches ramify to form a plexus which covers not only the more interior surface of the gonadial pouch, but also the surface adjoining the muscles. This plexus is extremely delicate and fine-meshed. The more exterior portion is continuous throughout the region of the gonadic pouches, the network arising from one nerve uniting with that which is in connection with an adjoining nerve. This network also joins the more dorsal fan-like branches of the rami viscerales ascendentes. This plexus is probably also continuous with that which is in connection with the intestinal canal, and it possibly extends beyond the anus. When the gonadic pouches are large and press against each other, the plexus on their more interior surfaces appears continuous and the origin of branches from the visceral rami is largely hidden. Figure 13 (Pl. 3) shows only the larger meshes of the plexus on the internal surface of a gonadic pouch, but the origin of these branches is illustrated. In Figure 10 (Pl. 2) the finer meshes of this plexus are shown under greater magnification. In methylene-blue preparations the threads of the gonadic plexus often appear faintly granular, indicating that they impregnate in a manner similar to the nerves of the inner abdominal plexus. Heymans et van der Stricht ('98) find the "peritoneal" plexus less rich under the sexual glands, but in this they probably refer only to the larger meshes. These authors do not figure the origin of branches running to the gonadic pouches, and give no detailed account of their innervation. Dogiel (:02) does not discuss the innervation of these organs.

The main stem of an ascending visceral ramus may usually be followed dorsad a distance equal to the width of one, two or even three myomeres from the ventral border of the side muscles, and then it breaks up into a large number of branches spreading out fan fashion over the inner surface of these muscles. In the anterior part of the body these fans are directed somewhat anteriad as well as dorsad (Pl. 4, Fig. 15), but the branches of the more posterior ascending visceral rami take in general a dorsad direction. If the wall of the branchial basket is lifted to disclose these nerves, the numerous branches of a fan often appear broken off abruptly in the middle region of the side muscles, and the distal broken ends are most frequently bent away from the muscles as though lying in a membrane not closely attached to the side muscles. This is no doubt due to the entrance of these nerves into the ligamentum denticulatum. As may be noted in Figure 15, *c* and *d*, the branches of one fan anastomose with those of an adjoining fan, thus forming a continuous network along the inner surface of the side muscles, and in the ligamentum denticulatum. This plexus may or may not lie dorsal to the gonadic pouches, depending on the size of the pouches. As has been mentioned, these fans join nerve threads which innervate the sexual glands.

From the plexus formed by these fans, certain branches pass upward along the ligamentum denticulatum (Pl. 4, Figs. 16, 17), while others ramify extensively in the "pocket" portions of this ligament. These nerves often present a fibrous appearance, as noted by Fusari; but this seems to depend on the time or manner of fixation. In other cases they appear dotted, with rough edges, the latter perhaps due to the deposit of a small quantity of coloring matter in the surrounding tissue. The nerves to the branchial basket pass upward along the ligamentum denticulatum in numerous small threads, and enter the basket not far from its dorsal region of attachment. The nerves in the "pocket" portions of the ligament run more or less longitudinally (Fig. 17), while those in the portions attached to the primary bars take a nearly vertical direction. As may be noted in Figure 17, nerves pass to the primary and secondary bars from various portions of the ligament. Figures 14 (Pl. 3) and 19 (Pl. 4) show the nerve threadlets of the primary and secondary branchial bars, and the connecting threads passing along the cross-bars. These figures, drawn from methylene-blue preparations, show an interesting likeness to Dogiel's Figur 17*a*, taken from a Golgi preparation. In the course of treatment after impregnation with methylene blue, the branchial epithelium sloughs off in large measure, exposing the nerve threads. The nerves which pass dorsad along the primary and secondary bars lie at first on the more exterior (lateral) surface of the branchial basket. As the threads ascend, they gradually shift toward the interior, and a greater number of threads are often found on the more posterior portion of each primary bar. Near the top of the primary bar these threads pass into a plexus lying between any given bar, and the next posterior secondary bar (Fig. 20). It is quite possible that as many fibers ascend on the anterior side of the bars to join this dorsal plexus, but the specimens observed did not show as great a number. Nerves passing along the secondary bars join this dorsal plexus with a smaller number of threads. In the middle region of the branchial basket, and in its ventral portion, the nerve threads lie on its more external surface. The nerves of the primary bars are loosely placed, and form an irregularly meshed network, the threads of which are not noticeably arranged lengthwise of the bar (Figs. 17, 19). On the secondary bars (Pl. 3, Fig. 14), on the contrary, the slightly knotted nerve threads form a plexus consisting of numerous long, longitudinally arranged threads, connected by short branches. This plexus appears to be rather smoothly drawn over the surface of the bar, in contrast to that of the primary bars. The knotted appearance of threads on the secondary bars may be an artificial condition. Nerve threads crossing from one bar to another along the cross-bars form a plexus over the latter in their course. At least, in the region dorsal to the ligamentum denticulatum fibers pass from primary to secondary bars at other points than the cross-bars, thus adding to the elaborateness of the branchial plexus.

I find a large number of the previously mentioned special nerve cells, which were first described by Dogiel (:02) as connected with nerves of the branchial basket. Dogiel figures these cells as they appear in Golgi preparations, and states that in methylene-blue preparations he noted large round or oval nuclei on certain nerve branchlets of the branchial basket, which he identifies as the nuclei of the cells found in Golgi preparations of the branchial region. These "nuclei" are shown in his Figur 16, taken from a methylene-blue preparation. This figure shows a condition frequently present in my methylene-blue preparations. Comparatively smooth lines, darkly colored with methylene blue, run parallel to the branchial bars, but do not appear to be connected with the evident nerves of the pharynx. Possibly these may be blood vessels; at any rate the nerve cells (Figs. 19, 21) which are impregnated with methylene blue in my preparations are not connected with these lines, but with the branchial plexus. Figures 19, 21, and 30 show these cells in methylene-blue preparations, and in a Golgi preparation. They correspond in shape and number of processes to those described by Dogiel. The nuclei of the cells impregnated with methylene blue are often difficult to discern, as is frequently the case in other nerve cells impregnated more or less with methylene blue. This led to the suggestion that these bodies might themselves be nuclei. Upon measurement of these so-called cells, and those impregnated after the method of Golgi, it was found that the size in either preparation was practically the same, the bodies in methylene-blue preparations being if anything, a little rounder and fuller. In the Golgi preparation illustrated (Fig. 30) a lighter nucleus is plainly visible in the multipolar cell. These cells are present on both primary and secondary bars, and are present at least in the ventral and side regions of the pharynx, on its external surface. In the ventral region these cells are distributed in connection with the elaborate plexus which spreads over this portion of the branchial basket.

There are certain branches of the rami viscerales ascendentes which are connected with the fan-like branches of these rami, but apparently do not have the same ultimate destination (Fig. 15, *e*), since they are lost to view in the region between the muscles and the ligamentum denticulatum. Heymans et van der Stricht ('98) figure nerve branches of the ascending visceral rami (Pl. VIII, Fig. 26, *N D V.*), which they designate as digestive and vascular, but the destination of such branches has never been satisfactorily determined. It is certain, however, that further experiments with methylene-blue impregnation and other methods, will reveal many additional complications in the distribution of the visceral nerves of Amphioxus. The investigations up to the present time establish the fact that the visceral rami of the dorsal nerves are elaborately connected throughout the atrial region, and that multipolar nerve cells occur in connection with these visceral nerves.

E. Nerves Posterior to the Atriopore.

Dogiel (:02) has described in detail the main cutaneous branches of these nerves, and illustrates the larger branches in his Figures 2 and 3. He notes the presence of "rami communicantes" connecting two adjacent ventral rami of dorsal nerves in their course over the side muscles of this region, but does not limit the occurrence of such connecting branches to the posterior portion of the body.

In the specimens which I have observed, the cutaneous branches in this region are more numerous than represented by previous authors. Rich plexuses are frequently present both between the dorsal rami and between the ventral rami of these nerves on the same side of the body (Pl. 6, Figs. 34–36). These plexuses will be described in connection with the sensory nerve endings. A "ramus communicans" was frequently found in this region, as well as in other parts of the body. The elaborate branching of the last dorsal nerve of Branchiostoma caribaeum is illustrated in Figures 31 and 33 for the right and left sides respectively. This pair of nerves is exclusively cutaneous, and its branches are more numerous than the figures by previous authors indicate. The most posterior dorsal nerve is posterior to the last ventral root, and, according to Hatschek's interpretation of the relation of the nerves to the myomeres, belongs to the preceding myomere. He states that each dorsal nerve belongs to the myomere anterior to the myoseptum through which it passes, and that the ventral root innervating this myomere appertains to this dorsal root.

Little attention has been previously given to visceral branches in this region. Heymans et van der Stricht ('98, p. 43) note in Golgi preparations apparent nerve fibers on both the parietal and visceral mucosa faces posterior to the abdominal pore. They were unable to find the origin of these nerves, or to trace their course, but suggest that they may come from the "abdominal" visceral nerves (Hatschek), or, more probably, as an extension of "thoracic" longitudinal nerves into the "abdominal" cavity. These authors seem to apply the term "abdomen" to the region containing the intestinal canal. They also note in Golgi preparations nerve fibers supplying the surface of the intestine, and (p. 44) branches from certain dorsal nerves (14th to 16th from the tail end) of the left side of the body supplying the anus. A branch from the right 13th (from the tail end) appears to have the same destination. Dogiel (:02) states that the dorsal nerve pairs from XL, or XLI, to LIII, or LIV, inclusive, give off ascending visceral branches, which bend around the ventral border of the side muscles, or penetrate a myoseptum to reach the interior. Beginning with nerve XLI or XLII, these visceral branches divide into a great number of branchlets, which weave around the whole rectum reaching to the anus. A fine plexus is formed in the walls of the anus from exceedingly fine and slightly varicose branches of nerves LI, LII and LIII. Dogiel does not state that the nerves of one side are more concerned in the innervation of the anus than those of the other. It is uncertain whether or not he finds visceral branches from the nerves succeeding nerve LIV; his figures surely do not illustrate such branches. His Figures 19*a* and 19*b* show ramifications of the visceral branches of nerves XLII to XLVI inclusive. These extend on the inner surface of the side muscles from their ventral border a short distance dorsad. Both figures are drawn as seen from the exterior.

In the specimens I have examined, occasionally visceral branches were apparently lacking to certain dorsal nerves, but, as Dogiel has observed, the manner in which these penetrate to the interior may be such that a view from the exterior fails to give any evidence of their presence. Examination of a large number of specimens disclosed visceral branches to practically all the nerves of this region from XL to LXI, inclusive. However, visceral branches could never be discerned from the exterior for all of these nerves in a single specimen. In one case nearly all dorsal nerves as far as nerve LX showed visceral branches. I have never observed visceral branches in connection with nerves LXII, LXIII or LXIV (when 64 nerves are present). The innervation of the anus, which lies on the left side, is usually supplied by branches of *left* nerves LI–LIII, in both species. Variation occurs here, and it is probable that in Branchiostoma caribaeum more anterior nerves may occasionally innervate this structure. Frequently only two of the nerves mentioned above give off branches which weave about the anus. Right nerves LI, LII and LIII often show branches penetrating to the interior, but these could never be traced to the anus.

In methylene-blue preparations suitable for dissection, the intestine is rarely in favorable condition for study, although the main visceral nerve branches impregnate particularly well. Such preparations cut along the dorsal median line, and laid open with the internal surfaces uppermost, proved very interesting when studied with the aid of strong light. Figure 32 (Pl. 6) is drawn from a specimen dissected after this fashion. It is evident from this figure that many more nerve branches penetrate to the interior than can be observed from an external view. These branches appear smooth and darkly stained for a short distance after they reach the interior, and their finer branches ramifying over the base of the ventral fin have a similar appearance. As the visceral branches ascend and ramify on the internal surfaces of the muscles, they are more lightly colored, and often flatten out into comparatively broad bands, which appear loosely confined. On the right side may be noted branches which probably supply the posterior extension of the atrium. The nerves ramifying over the base of the ventral fin are not to be confused with the cutaneous supply. The latter may be seen in a specimen dissected and examined in the manner just described, but they lie at a much lower focus (i. e. nearer the surface). A few nerves were observed forming a network over the inner surface of the side muscles posterior to the anus, thus accounting for at least a part of the visceral nerves observed from the exterior in this region. The nerve supply for the intestine probably reaches the latter through the membrane (mesentery) which holds it in place beneath the notochord. This corresponds to the manner in which a nerve supply reaches the pharynx. The presence of the post-atrial extension on the right side of the body in this region, may bring about modifications in the arrangement of the visceral nerves of that side. Certain abruptly ending branches, indicated at *c*, *c*, in Figure 32, may be the result of imperfect impregnation, or have a significance not yet determined. It seems certain that a network of nerve threads similar to that noted in the branchial region spreads over the internal surfaces of the muscles posterior to the atriopore.

In certain transverse sections of the tail region, stained with Mallory's differential stain, a pathological condition was evident, involving nearly all the structures of one myomere, and present to a slight extent in an adjoining one.

Considerable variation was noted in the total number of dorsal nerve pairs in each species. In Branchiostoma lanceolatum the number counted most frequently was 63; while in B. caribaeum the number appeared to be between 56 and 60.

F. Spinal Ganglia.

Dogiel (: **02**) found special structures in connection with the dorsal nerves of Amphioxus which he interprets as spinal ganglia. These bodies appeared in specimens immersed from 3–6 hours in a dark blue or violet mixture either of a 1% methylene blue, or of a saturated solution of toluidin blue, in normal salt solution. They could also be seen in gold-chloride preparations when the lemon-juice and formic-acid method was used. These so-called ganglia consist of groups of from 3 to 7 round, oval or pear-shaped elements, situated at the place of exit of each dorsal nerve from the myoseptum, or near the proximal end of the dorsal or ventral ramus where it gives off small branches; in addition to these, similar, though smaller, bodies may be found even as distant as the ventral border of the trunk muscles. These structures are illustrated in Dogiel's Figures 20, 21, 22*a*, 22*b*, 22*c*, 22*d*, and 23.

By using Dogiel's methods I was able to observe these structures in methylene-blue and in gold-chloride preparations, and also found them in specimens fixed in weak osmic acid and stained with picro-carmine. They appeared not only at the places indicated by Dogiel, but by using strong light for study of the preparations, they were found at almost any point in the course of the dorsal nerves. In methylene-blue preparations the size and number increased with the length of immersion. These bodies were of varying size, in some cases quite large, while by using the higher magnifications on portions of tissue mounted under a cover-glass, similar structures of minute size could be seen in connection with the smaller nerve branches. Strong light revealed great numbers of these structures in connection with the nerves of the thicker parts of the body. The methods mentioned above always cause a marked enlargement of the ampulla-like dilation at the posterior end of the neural tube, and a general increase of size in the tube itself. These facts led to doubts as to the reliability of such methods, and numerous experiments were made in the spring of 1905 to determine the nature of these bodies. Dogiel's methylene-blue method, employing normal salt solution instead of sea-water, causes general swelling of the tissues, and in some cases death occurs, followed by certain post-mortem changes, before these bodies appear. In material "fixed" in weak osmic acid and stained with picro-carmine these structures were frequently present, or, if not, I was able at will to make them appear by pressing lightly on the cover-glass over the mounted specimen. In some cases the pigment of the neural tube had passed out into the swellings in the course of treatment with chemicals, or upon pressure, and contrasted brilliantly with the light stain. An excellent illusion was sometimes produced by the presence of well stained nuclei, which are either pushed out from the cord, or are sheath nuclei. The weak osmic acid has little effect on tissues beneath the surface, and the subsequent washing in water causes actual maceration. This would account for all manner of artifacts. The specimens treated according to the gold-chloride method mentioned above, show general distortion, and many structures are so displaced as to make such preparations quite unreliable for study. These bodies, if present in methylene-blue preparations, will persist when the preparations are preserved in the mixture of glycerine and ammonium picrate; but when fixed in ammonium molybdate and *dehydrated* they usually disappear. Similar structures were never found in well preserved and sectioned material. There seems, therefore, but little doubt that these so-called "ganglia" are artifacts. Since writing the above account, Johnston's (:**05**) paper was noted, in which he also concludes that these structures are of artificial formation. Johnston finds a small proportion of the ganglion cells of Amphioxus in the nerve cord, and the remainder in the dorsal roots, located as far as, and beyond, the place of division into dorsal and ventral rami.

G. Structure of the Dorsal Nerves.

In methylene-blue preparations of the dorsal nerves, darkly stained fibers can often be traced from the neural tube, or place of exit of the nerve, through the myoseptum to the ventral border of the lateral muscle. These fibers are more or less separated from one another, and are not of equal size. Usually a single fiber cannot be traced for this entire distance. Fibers can frequently be discerned in the rami cutanei ventrales which give these branches a darkly colored appearance. I have often traced one, two or three fibers into the common basal trunk of the descending and ascending visceral branches, but was unable to trace distinct fibers into either of those branches. As has been noted, however, these nerves sometimes present a fibrous appearance. Figures 40 (Pl. 7) and 22 (Pl. 5) show fibers of at least two sizes entering the dorsal nerve roots, the smaller fibers being by far the most numerous. This agrees with the observations of Johnston (:**05**). The different structural appearance of the visceral nerves has already been noted. This difference is particularly evident in methylene-blue preparations, and may be observed in all branches of dorsal nerves innervating deep-lying structures. It is entirely possible that structural distinctions will be established between the visceral nerves to different organs.

H. Sensory Endings of Dorsal Nerves.

This subject has long attracted investigators, and its study has brought forth a great variety of opinion and suggestion. Quatrefages (**'45**) described peripheral nerves ending in small ovoid bodies (the corpuscles of Quatrefages), which he suggested represent special mucous organs. Kowalewsky (**'67**) believed that the sensory nerves end in epithelial cells. Owsjannikow (**'68**) occasionally saw what appeared to be a nerve fiber connecting with a cylindrical epithelial cell. He also noted what he calls a nerve-net, lying in the deepest layer of the skin, a region well supplied with connective tissue. Reichert (**'70**) describes certain cells in the epithelial layer of the skin, which bear a spine-like external process. He designates these cells as "thorn" cells. Langerhans (**'76**) found no branches uniting two dorsal nerves, or any exchange of fibers. All dorsal nerves branch in simple tree fashion. He believes that nerve plexuses are lacking in all parts of the body, except at the mouth border. He found no vestige of a fine end plexus such as Marcusen describes, holding that Marcusen was deceived by connective-tissue fibrillae. Langerhans describes special small cells lying irregularly between the cylindrical epithelial cells of the skin. These special cells have a small body and large oval nucleus; they lack a cuticula (limiting or basement membrane), and often possess a thread-like process at the internal end. On the external surface each cell bears a long, stiff hair, which sometimes has a thickened base. These special cells are particularly numerous in the head region, but may be found on all parts of the body. The hairs may be seen on living animals, but never show motion. The finer dermal nerve branches pass through small canals in the so-called limiting membrane of the skin, each of which is located where two small fissures in the membrane cross each other, usually at right angles. After a short sub-epithelial course, these fibers unite with hair-bearing cells, which Langerhans declares to be the endings of the cutaneous nerves. These nerves are not in relation with the ordinary epithelial cells, in fact, there are not enough nerve branchlets to permit union with each such cell.

Rohon (**'82**) states that the greater number of nerve branchlets run out into the cutis and end in tufts. Fusari ('89) says that the cutaneous nerves divide into fine branches immediately beneath the cuticula (the limiting membrane of Langerhans), and that anastomoses are frequent in the skin of the ventral region, but rare on the sides and dorsum. The branches in the ventral region pass into fine free-ending fibrillae, or before ending show a spindle-shaped, nucleated enlargement. In all other regions (with the exception of the mouth) nerves, penetrating the cuticula, appear to terminate after a short course in free endings, though they can be traced to the bases of epithelial cells. Fusari is uncertain, however, whether there is any connection between these nerve fibers and the cells. He saw the sensory cells of Langerhans, and though inclined to his views, contends that a connection with the nerves is not demonstrated. He believes that the description of the nerve-net given by Marcusen is based on a peritoneal rather than a dermal plexus. Heymans et van der Stricht ('98, p. 33) regard the so-called sense-cells of Langerhans as merely compressed and flattened "cyclinder" cells, bearing no special relation to the nerves. These authors saw branches of a dorsal nerve form among themselves a plexus on the border of the fins; but they never found the plexuses mentioned by Fusari as frequent in the ventral region. According to these writers the nerve fibers ramify under the cutaneous epithelium, and terminate between, and perhaps within, ordinary epithelial cells. They found no special sense-organs in the skin, in connection with nerve fibers.

Retzius ('98) is convinced that cutaneous fibers end free at the bases of epidermal cells. He saw two kinds of cells in the epithelium, one smaller than the other, but neither bore a thread- or spine-like process. He found no direct connection between these cells and nerve fibers, nor could he discover any true peripheral sense-cells connected with nerves. Dogiel (:02) states that the "Nervi cutanei dorsales (laterales et ventrales)" are pure sensory branches without any motor fibers. These gradually divide in their course to the superficial layers of the skin, continually giving off branches of various lengths and thicknesses, which anastomose with similar neighboring branchlets to form a more or less elaborate plexus, his "Grundgeflecht." This plexus is especially plain, he says, in the skin of the ventral region. His further description is as follows: The branches from this plexus ultimately reach the homogeneous layer of the skin immediately beneath the epithelium, and can be observed in good preparations to pass through small canals in this layer. Each then divides into three or four or more fine branchlets, which spread out radially. These branchlets, which lie immediately beneath the epithelium, in turn divide repeatedly in this region, giving off fine, varicose threads, which form a close-meshed plexus. This is designated by Dogiel as the "sub-epithelial plexus." From this plexus fine threadlets penetrate between the epithelial cells, and there give off side branchlets, which twist about these cells and end between them; these threads were followed almost to the outer surface of the skin. Dogiel found such endings in the rostrum, in the middle region of the body, and in the tail. But in addition to these free nerve endings in the epithelium, Dogiel also saw in the epithelium what he regards as peripheral nerve cells; these occur in the rostrum, the tentacles, and the "head" region. He believes that they are scattered over the entire skin area. These cells have a spindle-shaped body, with a peripheral and a central process, and a large nucleus, nearly filling the thick part of the cell. The peripheral process is a short, thick cylinder or rod, which reaches close to the outer surface of the epithelium, and its end is either blunt, or somewhat pointed, but it never extends in the form of a thread beyond the free surface of the skin. The central process runs perpendicularly or obliquely toward the interior, and could occasionally be seen passing through the homogeneous layer of the skin and uniting with a nerve branchlet. Dogiel's Figur 28 presents a portion of the "sub-epithelial plexus" and its branches in the ventral region of the body, in connection with the well-known plexus formed from branches of the rami cutanei ventrales. His figures 29 and 30 show peripheral nerve cells in the skin of the rostrum and head. Dogiel also illustrates nerve-endings in the tentacles of the mouth.

In my own study of the sensory nerve terminations in Amphioxus a great number of methods were employed, including methylene-blue impregnation, the methods of Golgi, the various gold-chloride methods, picro-carmine staining, and sections of material fixed and stained in a variety of ways. As has been noted, the extensive arborescent appearance of the cutaneous branches of the dorsal nerves may be readily observed in methylene-blue preparations studied with the aid of strong light (Pl. 6, Fig. 34; Pl. 7, Figs. 37, 39). The arrangement of the sensory endings must depend largely on the distribution of the finer terminal nerve branches. The extreme readiness with which Amphioxus reacts to tactile stimuli applied to any part of the body, indicates a rich supply of cutaneous nerves. The sensory nerve branches reaching to the epithelial layer of the skin are most readily followed in methylene-blue preparations. The best impregnations of these nerves were obtained by immersion of the specimens from 1½ to 1¾ hours. The subsequent exposure to air varied from 15 to 40 minutes. The specimens were fixed in ammonium picrate to which a little osmic acid had been added, or in ammonium molybdate and osmic acid. Direct sunlight, a Welsbach lamp, or a Nernst lamp were used in studying whole specimens.

The distal ends of the finer cutaneous nerve branchlets are often noticeably directed toward the exterior (Pl. 7, Fig. 37). By focusing on the plane in which the exterior ends of these nerve branchlets lie, it may be observed that the surface of the cuticula is broken by numerous small clefts crossing each other either at right angles, or in the form of an X. By focusing downward upon a dark spot at the place of crossing, it can be determined that this spot is the end of a nerve branchlet, which can be followed to its place of union with one of the larger branches. These numerous small, exteriorly directed branchlets for the most part, therefore, penetrate the sub-epithelial so-called cuticula (better basement membrane) through the small canals first described by Langerhans. These small branchlets are more numerous than either Heymans et van der Stricht ('98), or Dogiel (: 02) have figured. They were not always evident, and it was frequently impossible to demonstrate a basement membrane, which seems to indicate that this structure sometimes sloughs off with the epithelium. Figures 34 (Pl. 6) and 39 (Pl. 7), which show these exteriorly directed small branchlets in considerable numbers, also show other characteristic cutaneous nerve branches. I refer to large branches, lying close to the muscles, which may be noted connecting the branches of two or more dorsal nerves in their course over the side muscles (see also Pl. 6, Fig. 36, p_1 and Pl. 7, Fig. 38, p_1). Beside these connecting branches, there are, lying at a higher focus, finer plexuses, which connect the small, exteriorly directed branchlets. These finer plexuses may also be seen in Figures 35, p_2 and 38, p_2. In methylene-blue preparations this finer plexus is usually the most superficial nervous structure visible. The branches of the deeper plexus are usually of considerable size, and fewer in number than those of the more exterior plexus, and their meshes are larger than those of the outer plexus. As has been noted, the deeper plexus lies close to the muscles, and its branches may even run beneath other large branches of the dorsal nerves. This plexus between the ventral rami of dorsal nerves was found distributed over the side muscles in nearly all parts of the body. The finer plexus is composed of slender threads, forming close meshes; it was found in the regions where there is a coarser plexus, and also in the ventral fin, and between the dorsal rami of dorsal nerves. The and therefore may lead to much confusion. In the ventral region, where the tissues are transparent, connective-tissue threads may be readily discerned in gold-chloride preparations, weaving about the nerves, and running out from them in various directions like guy-ropes. These threads thus furnish an excellent pitfall for the observer. Where exceedingly fine nerve branchlets and connective-tissue threads are associated with each other, one may well hesitate to decide between them. The bodies (*c*) connected with the fine threadlets shown in Figure 24, all lie in the same plane with the threadlets, whereas the epithelial cells and special cells (*g*) are more superficial, being immediately exterior to these threadlets. The latter may be terminal nerve branches, interweaving at the bases of the epithelial cells, and sending branches between them, but evidence furnished by some other method is needed for corroboration. If these threadlets are nerves, they are probably connected with the finer plexus noted in methylene-blue preparations.

The special cells (*g*) previously mentioned are shown in Figure 42 (Pl. 7) under greater magnification. This figure illustrates the external surface of skin overlying the side muscles near the dorsal fin. This preparation was impregnated with gold chloride after Ranvier's method. In preparations of this kind the ordinary epithelial cells are light purple in color, while certain differentiated cells lying between them appear red, are somewhat smaller, and are oval or circular on their external faces instead of polygonal. One or two slightly modified epithelial cells (*s*) are usually associated with each special cell. These accompanying cells assume a darker purple color than the ordinary epithelial cells. The special cells (*g*) and the modified epithelial cells (*s*) were both present in certain methylene-blue preparations, distributed in the rostrum and anterior portion of the body as far as nerve XVI. Unfortunately, favorable preparations of the skin of more posterior portions of the body were lacking. In these methylene-blue preparations the special cells assume a dark blue color, while the modified epithelial cells are only slightly darker colored than the ordinary epithelial cells, and present a granular appearance.

Changes in focus show that the diameter of these special cells is slightly less at the surface than at a short distance toward the interior. The most striking feature, however, of these cells is a rather long, stiff hair-like structure attached to the outer surface of each cell. In gold-chloride preparations this hair-like structure is colored black, or a very dark purple. In methylene-blue impregnations it appears dark blue. As shown in Figure 42 (Pl. 7), this "hair" is thicker at its distal than at its proximal end, and often bears a knob-like termination. Two apparent points of attachment to the cell are frequently visible; one of these occurs at almost any point on the outer surface of the cell, while the other is at one side of the cell, or perhaps between two cells. While these special cells may possibly correspond to the "thorn" cells (Stachelzellen) noted by Reichert ('**70**, p. 756), this stiff hair is much too long for such a comparison, nor does it resemble a spine, because of its thickened distal end; neither does it resemble any terminal nervous structure. One interesting feature is the varying length of such "hairs," and their absence from many of the special cells. The "hairs" were nearly always comparatively short in methylene-blue preparations. I suggest that these special cells may be gland cells, and that the "hairs" are hardened exudations adhering to the outer surface. Glands certainly exist in the skin of Amphioxus, and probably receive a nerve supply from the cutaneous plexuses. Attempts were made on hardened material to color the "hairs" with special stains for glands, but none of the material was properly fixed to make such tests of value. If these peculiar cells are gland cells, it is still uncertain whether sensory cells are present in the epithelium of all parts of the body.

Sensory cells are clearly present in the tentacles, and on the mouth border, as described by Dogiel (:**02**, p. 192–195; Fig. 10, 31, 32). These cells impregnated successfully in certain of my methylene-blue preparations, and nerve fibers could be traced in the tentacles to the region of these cells. I was unable, however, to determine absolutely the connection between the two which in all probability exists in the form already shown by Dogiel. The long distal process of the end-cells in the papillae of the tentacles, figured by Dogiel was clearly evident in my preparations.

VENTRAL NERVES.

Owsjannikow ('**68**) describes nerve roots arising from the spinal cord at different levels. Although Stieda ('**73**) thought it probable that this author saw ventral nerve roots, he himself was the first to describe the ventral roots clearly; he notes that they do not lie in the cross-section plane of the dorsal roots, and he found no actual union between them and the dorsal roots. Langerhans ('**76**) saw only dorsal roots, and regarded them as carrying motor fibers. Schneider ('**79**) described each ventral nerve root as arising by several processes (each leaving the nerve cord by a separate orifice), which unite into a flat bundle; this soon spreads out, a small portion going dorsad, and a larger part ventrad. When the fibers reach the muscle layer they bend posteriorly and coalesce with the free margin of the muscle-plate. As the fibers approach the border of the muscle-plate, they become cross-striated. Schneider proposed the theory that the real motor nerves extend outward only as far as the sheath of the spinal cord, and that each muscle-plate sends a process to the spinal cord and receives its innervation there. Balfour ('80) denies the presence of motor roots. Rohon ('82, p. 14, 54) states that the ventral roots arise from multipolar ganglion cells, in part pigmented, which lie on both sides of, and in part ventral to, the central canal of the cord. He finds no morphological relation between dorsal and ventral roots outside the spinal cord and is entirely in the dark as to how the motor nerves end. Rohde ('88) thinks, with Schneider, that the motor fibers are probably in part direct processes of the muscle fibrillae; but he is uncertain whether there is a direct connection between the motor fibers and the nerve elements of the neural tube. Fibers in the ventral roots were followed centripetally to a delicate membrane on the inner side of the corda sheath, where they were believed to divide into very fine fibers, which, however, could not be traced in the spinal cord. Fusari ('89) finds, as did Schneider and Rohde, most of the ventral-root fibers cross-striated; but some of them are not striated, exhibit enlargements, and stain black by the Golgi method. Retzius ('91) notes in each ventral root an anterior division composed only of varicose fibers, and a posterior, somewhat smaller division carrying fibers mostly without varicosities. Each fiber of the posterior portion bears an oval body a short distance after its exit from the spinal cord. Retzius is unable to decide whether these are sheath nuclei, or of some other nature. Toward the spinal cord motor nerve fibers were followed singly into the outer layer of the cord, where they enter a low, granular mound; but beyond this they cannot be followed. In these mounds the fibers often bend in hook-fashion. Retzius thinks that the connection of motor fibers with central ganglion cells, which he does not doubt, cannot be very direct. Fibers occasionally divide dichotomously, but there are no special nerve endings. Especially characteristic of these fibers is their varicose-granular condition. Owing to the abundance and transverse elongation of the granules, they give a cross-striated appearance to the fibers, probably the cause of Schneider's erroneous views, for these are true nerve fibers, and not of muscular nature. He is inclined to accept Rohde's suggestion that the *transverse* muscles are innervated by fibers carried by the dorsal roots.

Heymans et van der Stricht ('98) believe that the presence of a pair of dorsal nerves behind the last ventral pair indicates the disappearance, or non-appearance, of a posterior myotome, and on this basis regard each dorsal root as corresponding to the following, not the preceding (as Hatschek states), ventral root. The division of a root into dorsal and ventral portions (Retzius) is based on an optical illusion, or on the effect produced by sections. The motor fibers, studied largely on Golgi preparations, penetrate between muscle plates and reach the peripheral zone of the myotome; they sometimes branch. Both ascending and descending fibers show varicosities, consisting of regular and of irregular swellings. The regular swellings are probably nuclei, but those of irregular occurrence are artifacts. Motor fibers show definite terminal bodies, which are flattened and conical, of spatulate form; they are perhaps to be considered as motor plates of the cylindrical muscle fiber, upon which the terminal nerve fiber inserts itself, not perpendicularly, but laterally. From the number of these terminal plates the authors believe that each muscle fiber is innervated. The nerve fibers of the ventral roots are nonmedullated, and thicker than those of the dorsal roots. The striated fibers in ventral roots are not nerve fibers, but muscle fibers, deflected from their usual course.

Dogiel (: **02**) impregnated motor fibers and end-plates with methylene blue. He finds that the typical motor nerve ending is not a plate, but a flattened cone, the basal surface of which lies on the surface of a *muscle* plate. The cones appear larger in Golgi preparations than in those impregnated with methylene-blue, because in the former case silver is deposited to some extent in the muscle as well as in the nerve. Dogiel finds motor fibers penetrating (centripetally) the ventral part of the nerve cord in more or less thick bundles. These fibers arch backward, and can be followed for some distance. Certain fibers bend downward under the central canal, either from right to left, or vice versa, and so constitute a kind of commissural fiber. Dogiel could not follow fibers to cells, but states that they do not divide into fine threads, nor form a net on entering the nerve cord. Although he says that few varicosities are present in methylene-blue preparations, his Figures 43, 44*a*, and 44*b*, show frequent swellings in the motor nerve fibers. Repeated branchings of the motor fibers in their course toward the periphery are seen in his Figure 41.

In my own study the most successful impregnations of motor fibers were obtained by use of the rapid methods of Golgi, and by treatment with gold chloride after fixation with 10% formol. The methods of Golgi and their modifications have produced such varying results in the appearance of nerve fibers in vertebrates generally, that a great number of variations in length of time, temperature, etc., were employed in order to test, if possible, the reliability of the impregnations obtained. Several hundred specimens were subjected to these various modifications of the methods of Golgi, and enough specimens have been sectioned and examined to establish some definite facts. In general it was found that all animals must be cut into two or more pieces, depending on their size; since whole specimens, beside impregnating unsatisfactorily, usually show numerous artifacts. It is absolutely essential that the fluids employed be used in large quantities, and slight warmth was found advantageous in bringing about impregnation. A liberal amount of the silver nitrate should be used and changed at least once. The rapid methods were by far the most satisfactory, as shown by the greater number of fibers impregnated, and the freedom from artifacts. Dogiel states that varicosities in the motor fibers are constantly present in Golgi preparations. The accompanying figures (Pl. 5, Figs. 25–29; Pl. 8, Figs. 43–50) show that such is not the case. The difficulty in securing impregnations of motor fibers after they enter the neural tube suggests that their chemical composition may differ in that region. Modifications in the methods employed may hereafter bring results in this case, as in others.

The impregnations with gold chloride after fixation with 10% formol are comparatively free from distortion and artifacts, and are probably reliable as a basis for comparison. The impregnation of motor fibers and endings is somewhat uncertain with this method, but when successful the preparations are very satisfactory. The nerve fibers are not strikingly differentiated, but are favorable for study on account of their accurate fixation. These preparations show a tendency to fade after a considerable length of time. I did not succeed by this method in impregnating fibers distinctly as they enter the neural tube, but variations in the method may produce better results.

Strong light was particularly useful in studying the thick sections necessary for tracing the nerve fibers. The shrinkage in the surrounding tissues often causes wrinkling in fibers impregnated by the Golgi method (Pl. 8, Figs. 46, 47). These fibers also show a tendency to break, but the artificial nature of such breaks is usually evident.

The ventral nerves were studied in both species of Branchiostoma, and no differences were found which could not be readily explained by the uncertainty of the methods employed. The nerve fibers of the ventral nerves show such uniform smoothness in good preparations that it seems doubtful if any actual "varicosities" exist. The numerous smooth fibers illustrated in Figures 28, 29 (Pl. 5), and 46 (Pl. 8), and the uniform smoothness of fibers impregnated with gold chloride, certainly give grounds for such a conclusion. The small swellings sometimes present in my Golgi preparations are of irregular occurrence, and are usually found in whole specimens, or those impregnated according to the slow methods. It is true that occasionally structures resembling bipolar ganglion cells (Pl. 8, Fig. 50) are to be found toward the proximal ends of fibers in the ventral nerves, but one hesitates to regard these structures as cells when their presence here would indicate a marked variation from the typical condition. In Figure 50 an apparent nucleus is present in the cell-like body attached to the nerve fiber; but every investigator who has used the methods of Golgi knows what bewildering artifacts can be produced.

According to my preparations the motor fibers of Amphioxus leave the inner border of the spinal cord sheath (Fig. 25, Pl. 5) as rather slender, smooth fibers, apparently differing somewhat in size. These fibers are often curved or wrinkled in the sheath, and frequently bend sharply as they leave the covering of the neural tube. They are continued to the muscle border (Figs. 25, 26) as comparatively smooth threads, changing little in caliber. The spreading out of the ventral nerves has been described so often that it need not be taken up here. Upon reaching the side muscles certain motor fibers increase in size, while others do not change, depending apparently on their distance from the muscle fibers which they innervate. As a fiber approaches its distal end, it gradually thickens, and finally ends with the peculiar plate- or cone-like structure, first noted by Heymans et van der Stricht ('98). In contrast to the observations of Dogiel (:02), little branching is evident in the course of these fibers. Figures 28, 29 (Pl. 5) and 46 (Pl. 8), which show a large number of motor fibers, do not reveal branching. In Figures 28 (Pl. 5) and 43 (Pl. 8) single fibers may be traced from near the internal border of the muscles to a short distance from its exterior border and in one case (Fig. 43) to the termination of the fiber. These long, single fibers are significantly frequent in various parts of the myotome. Branching certainly occurs (Pl. 8, Figs. 48, 49), but my preparations do not reveal the extensive division in the region of the muscles described by Dogiel.

It may be noted in this connection that a greater number of nerve fibers are impregnated in the region of the muscles than in the neighborhood of the neural tube. This might be due to any one of several conditions; e. g., a fiber may change in character before it reaches the neural tube, thus presenting for impregnation different conditions in its two parts; or branching may take place more freely than my specimens indicate. Perhaps the mechanical tension on the nerves, between their exit from the neural tube and the nearest point of the muscle, due to the action of reagents, may destroy, or render unsuitable for impregnation, that part of the nerve.

The motor endings shown in Figures 27, 28 (Pl. 5) and 43–45 (Pl. 8) are probably fairly typical. They agree in size and form with those in gold-chloride preparations, and the surrounding muscle does not show the deposit of silver sometimes evident in material impregnated by the Golgi method. These endings were found in nearly all portions of the side muscles except the region adjoining the skin. They are present in considerable numbers a short distance from the exterior. Many endings were found dorsal to the neural tube, not far from the dorsal fin. I was unable to trace motor fibers into the neural tube beyond its sheath. No preparations of any kind showed motor fibers continuing in the neural tube in the manner figured by Dogiel in his Figur 45. In certain preparations, not cut in a true frontal plane, fibers appeared to end some distance inside the sheath of the neural tube, but on further study this proved to be an illusion, caused by the direction of the section. I am inclined to believe that the true course of motor fibers in the neural tube is as yet undiscovered.

BIBLIOGRAPHY.

Balfour, F. M.
'80. On the Spinal Nerves of Amphioxus. Quart. Jour. Micr. Sci., Vol. 20, pp. 90–91.
Dogiel, A. S.
: 02. Das periphere Nervensystem des Amphioxus (Branchiostoma lanceolatum). Anat. Hefte, Heft 66, pp. 143–211, Taf. 12–29.
Edinger, L.
: 06. Einges vom "Gehirn" des Amphioxus. Anat. Anz., Bd. 28, pp. 417–428.
Fusari, R.
'89. Beitrag zum Studium des peripherischen Nervensystems von Amphioxus lanceolatus. Internat. Monatsschr. f. Anat. u. Physiol., Bd. 6, pp. 120–140, Taf. 7, 8.
Goodrich, E. S.
: 02. On the Structure of the Excretory Organs of Amphioxus. Part I., Quart. Jour. Micr. Sci., Vol. 45, pp. 493–501, pl. 27.
Goodsir, J.
'41. On the Anatomy of Amphioxus lanceolatus; Lancelet, Yarrell. Trans. Roy. Soc. Edinb., Vol. 15, Part I., pp. 247–263, pl. 4, 5. *Also in* The Anatomical Memoirs of John Goodsir, edited by William Turner, etc., Vol. I., Edinburgh, 1868, pp. 371–393, pl. 1, 2.
Hardesty, I.
: 02. Neurological Technique, etc. xii+183 p. Chicago and London.
Hatschek, B.
'81. Studien über Entwicklung des Amphioxus. Arbeit. Zool. Inst. Univ. Wien, Tom. 4, pp. 1–88, Taf. 1–9.
Hatschek, B.
'92. Die Metamerie des Amphioxus und des Ammocoetes. Verhandl. Anat. Gesell., Sechste Versamml. Anat. Anz., Jahrg. 7, Ergänzungsheft, pp. 136–162.
Hesse, R.
'98. Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. IV. Die Sehorgane des Amphioxus. Zeit. f. wiss. Zool., Bd. 63, pp. 456–464, Taf. 24.

Heymans, J. F., et van der Stricht, O.

'98. Sur le système nerveux de l'Amphioxus et en particulier sur la constitution et la genèse des racines sensibles. Mém. couron. et mém. sav. étrang. Acad. Roy. sciences, lettres et beux-arts Belgique, Tom. 56, mém. 3, 74 pp., 13 pl.

Johnston, J. B.

:05. The Cranial and Spinal Ganglia and the Viscero-Motor Roots in Amphioxus. Biol. Bull., Vol. 9, No. 2, pp. 112–127.

Kowalewsky, A.

'67. Entwickelungsgeschichte des Amphioxus lanceolatus. Mém. Acad. impér. Sci. St. Pétersbourg, Sér. 7, Tom. 11, No. 4, 17 pp. 3 Taf.

Kupffer, K. von.

:03–05. Die Morphogenie des Centralnervensystems. Handbuch vergl. u. exper. Entwickelungslehre Wirbeltiere, Bd. 2, Theil 3, pp. 1–272. (Amphioxus pp. 1–12, 1903.)

Langerhans, P.

'76. Zur Anatomie des Amphioxus lanceolatus. Arch. f. mikr. Anat., Bd. 12, pp. 290–348, Taf. 12–15.

Leuckart, R., und Pagenstecher, A.

'58. Untersuchungen über niedere Seethiere. Arch. f. Anat., Physiol. u. wiss. Med., Jahrg. 1858, pp. 558–613, Taf. 18–23. (Amphioxus, pp. 558–569, Taf. 18.)

Lönnberg, E.

:01—.Pisces. Bronn's Klassen u. Ordnungen des Thier-Reichs, Bd. 6, Abth. 1. (Leptocardii Lief. 2–20, pp. 99–249, Taf. 1–12, 1902–1905.)

Macbride, E. W.

:00. Further Remarks on the Development of Amphioxus. Quart. Jour. Micr. Sci., Vol. 43, (N. S.). pp. 351–366, pl. 17.

Mallory, F. B.

:00. A Contribution to Staining Methods. Jour. Exp. Med., Vol. 5, No. 1, pp. 15–20. *Also abstr.* in Jour. Appl. Micr., Vol. 3, pp. 1036–1038.

Marcusen, T.

'64. Sur l'anatomie e l'histologie du Branchiostoma lumbricum. Costa (Amphioxus lanceolatus. Yarrell). Comptes Rendus Acad. Sci., Paris, Tom. 58, pp. 479–483. *Also transl. in* Ann. and Mag. Nat. Hist., Ser. 3, Vol. 14, pp. 151–154, 1864.

Müller, J.

'41. Mikroskopische Untersuchungen ueber den Bau und die Lebenserscheinungen des Branchiostoma lumbricum Costa, Amphioxus lanceolatus Yarrell. Monatsbericht über die zur Bekanntmachung geeigneten Verhandlungen. Königl. Preuss. Akad. Wissensch. zu Berlin, 1841, pp. 396–411. *Also sep.* 16 pp. [Berlin] 1841.

Müller, J.

'44. Ueber den Bau und die Lebenserscheinungen des Branchiostoma lumbricum Costa, Amphioxus lanceolatus Yarrell. Abhandl. Königl. Akad. Wissensch. zu Berlin. a. d. Jahre 1842, Physikal. Abhandl., pp. 79–116, 5 Taf. 1844.

Nüsslin, O.

'77. Zur Kritik des Amphioxusauges. Inaug.-Diss., Tübingen, 33 pp., 2 Taf.

Owsjannikow, P.

'68. Ueber das Centralnervensystem des Amphioxus lanceolatus. Bull. Acad. Impér. Sci. St. Pétersbourg, Tom. 12, pp. 287–302, 1 Taf.

Platt, Julia.

'92. Fibres connecting the Central Nervous System and Chorda in Amphioxus. Anat. Anz., Jahrg. 7, No. 9 u. 10, pp. 282–284.

Quatrefages, A. de.

'45. Mémoire sur le Système nerveux et sur l'Histologie du Branchiostome ou Amphioxus. Ann. Sci. Nat., Zool., Sér. 3, Tom. 4, pp. 197–248, pl. 10–13.

Rathke, H.

'41. Bemerkungen über den Bau des Amphioxus lanceolatus, eines Fisches aus der Ordnung der Cyclostomen. Königsberg, 38 pp., 1 Taf.

Reichert, C. B.

'70. Zur Anatomie des Branchiostoma lumbricum. Arch. f. Anat., Physiol. u. wiss. Med., Jahrg. 1870, pp. 755–758.

Retzius, G.

'91. Zur Kenntniss des centralen Nervensystems von Amphioxus lanceolatus. Biolog. Untersuch., N. F., Bd. 2, No. 2, pp. 29–46, Taf. 11–14.

Retzius, G.

'98. Die Methyleneblaufärbung bei dem lebenden Amphioxus. Biol. Untersuch., N. F., Bd. 8, No. 14, pp. 118–122.

Rohde, E.

'88. Histologische Untersuchungen über das Nervensystem von Amphioxus lanceolatus. Zool. Beiträge (Schneider), Bd. 2, Heft 2, pp. 169–211, Taf. 15, 16.

Rohon, J. V.

'82. Untersuchungen über Amphioxus lanceolatus. Denkschr. Akad. Wiss. Wien, Math.-Naturw. Cl., Bd. 45, 64 pp., 6 Taf.

Rolph, W.

'76. Untersuchungen über den Bau des Amphioxus lanceolatus. Morph. Jahrb., Bd. 2, pp. 87–164, Taf. 5–7.

Schneider, A.

'79. Beiträge zur vergleichenden Anatomie und Entwicklungsgeschichte der Wirbelthiere. Berlin, viii+164 pp., 16 Taf. (Amphioxus, pp. 1–31, Taf. 14–16.)

Schneider, A.

'80. Ueber die Nerven von Amphioxus, Ammocoetes und Petromyzon. Zool. Anz., Jahrg. 3, pp. 330–334.

Stieda, L.

'73. Studien über den Amphioxus lanceolatus. Mém. Acad. Impér. Sci. St. Pétersbourg, Sér. 7, Tom. 19, No. 7, 71 pp., 4 Taf.

Wijhe, J. W. van.

'84. Ueber den vorderen Neuroporus und die Phylogenetische Function des Canalis neurentericus der Wirbelthiere. Zool. Anz., Jahrg. 7, pp. 683–687.

Wijhe, J. W. van.

'93. Ueber Amphioxus. Anat. Anz., Jahrg. 8, pp. 152–172.

Willey, A.

'94. Amphioxus and the Ancestry of the Vertebrates. Macmillan and Co., New York and London, xiv+316 pp., and frontispiece.

EXPLANATION OF FIGURES.

Each drawing was outlined with an Abbé camera lucida. The numbers of the dorsal nerves are indicated by Roman numerals. Unless otherwise stated, dorsal is up in all figures.

ABBREVIATIONS.

N. B.— Unfortunately an entirely consistent system of lettering figures could not be adopted owing to the incorporation of the provisional lettering in the drawings themselves. With the reduction in size accompanying the reproduction of the figures, many of these letters are extremely small. For explanation of all letters not embraced in the following list, the reader may consult the explanation of the Figure on which the letters occur.

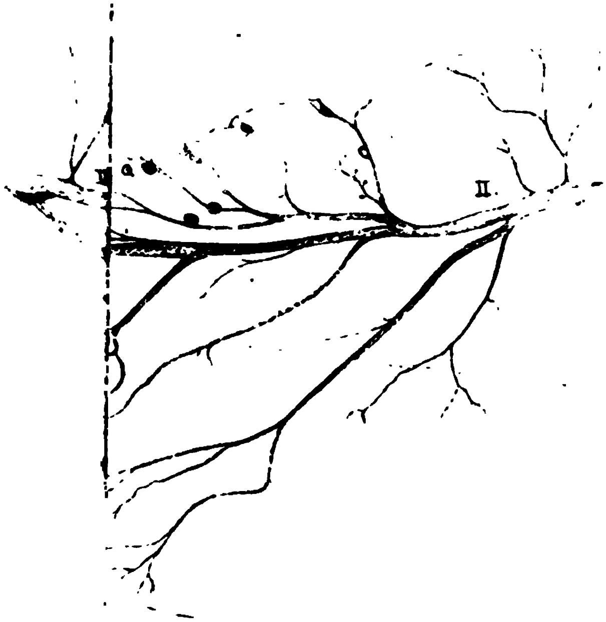
ant.	Anterior.
b. n.	Band nerve of velum.
brs. gon.	Gonadial pouch.
ch.	Chorda dorsalis.
dx.	Right.
e. p.	End-plate.
e. s.	Eye-spot.
i.	Nerve branches to inner mouth plexus.
i. a. p.	Inner abdominal plexus.
l.	Lateral.
l. d.	Ligamentum denticulatum.
m.	Muscle.
m_1, m_2, m_3.	Muscle of myomeres 1, 2, 3 etc.
marg. l.	Lateral margin.
marg. m.	Median margin.
med.	Median.
m. v.	Ventral margin of muscle.
my'sep.	Myoseptum.
n.	Nucleus.
n. t.	Neural tube.
o.	Nerve branches to outer mouth plexus. In Fig. 2, *o* is olfactory pit.
par. go.	Wall of gonadium.
post.	Posterior.
Q.	Cells of Quatrefages.
r. c.	Ramus cutaneus.
r. c. v.	Ramus cutaneus ventralis.
r. d.	Ramus dorsalis.
r. v.	Ramus ventralis.
r. v. a.	Ramus visceralis ascendens.
r. v. d.	Ramus visceralis descendens.
sin.	Left.
$t.^1$	Velar tentacle, small.
$t.^2$	Velar tentacle, large, central.
v.	Velum.

PLATE 1

All figures are from methylene-blue preparations of *Branchiostoma caribaeum*, w
the epithelium of the skin is not present.

Figure 1. Nerves of the right side of the rostrum. × 93.
Figure 2. Nerves of the left side of the rostrum. × 93.
Figure 3. Nerves of the right side of the rostrum. A few nerve branches i
the ventral region are not shown. × 93.
Figure 4. Nerves of the left side of the rostrum. × 93.

LEHMANN-PE PLATE I



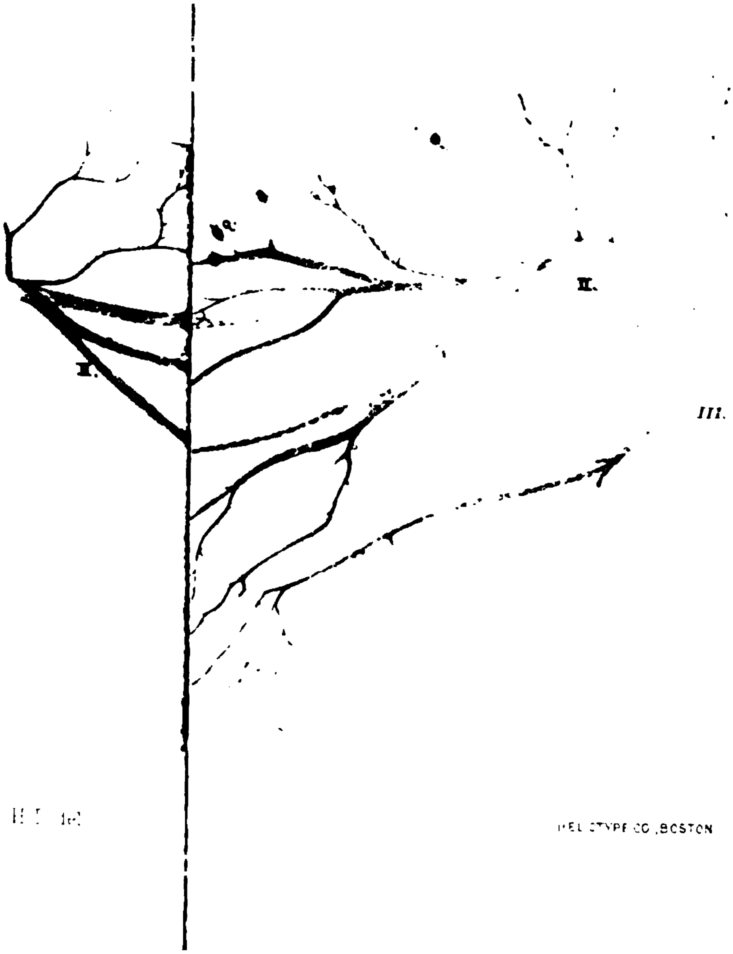


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PLATE 2.

All figures are from methylene-blue preparations.

FIGURE 5. Branchiostoma caribaeum. Nerves of the right side of the rostrum. The epithelium of the skin is not present. Numerous cells of Quatrefages, *Q*, *Q*, are shown. × 92.

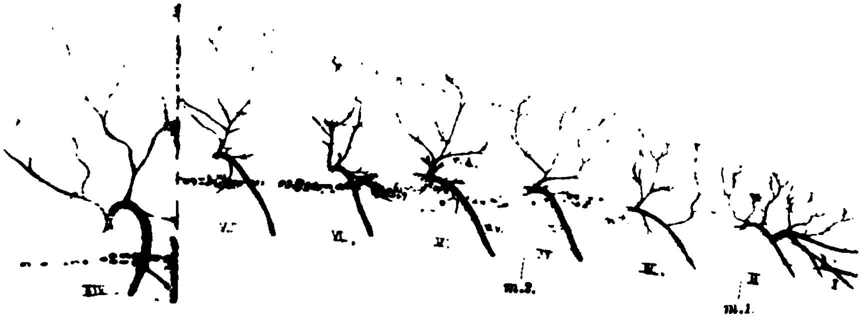
FIGURE 6. B. caribaeum. Anterior dorsal portion of a transparent specimen, showing the exit of the anterior dorsal nerves from the neural tube on the right side of the body. The branches of the dorsal rami of these nerves are drawn. Nerve II shows two roots. The places of division of these nerves into dorsal and ventral rami are also shown. × 32.

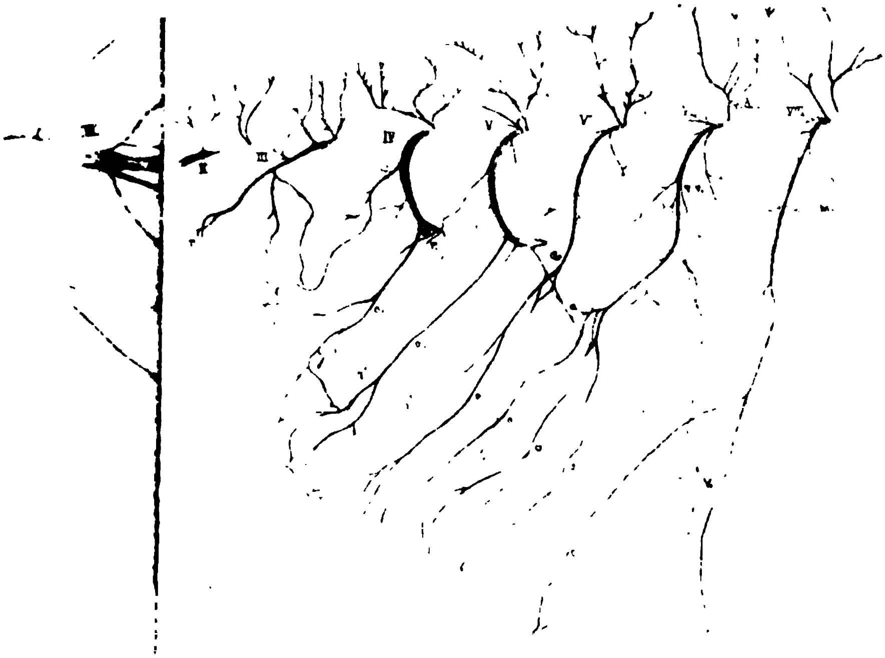
FIGURE 7. B. caribaeum. The main branches of the dorsal nerves of the buccal region, on the left side of the body. The branches *r*, *r*, pass to the right side of the body; *a*, *a*, indicate places of anastomosis. The branches *o*, *o*, form part of the outer mouth plexus; those marked *i*, *i*, form part of the inner mouth plexus; *v*. band nerve of velum. × 34.

FIGURE 8. B. lanceolatum. Anterior is up. The innervation of the velum. The epithelium of the velum is not present. The velar tentacles are indicated by t^1, t^2; *a*, *a*, point where one velar nerve band was cut; *b*, *b*, similar point in another cut nerve band; *b′*, the cut nerve *b* near the point where it diverges from δ. For nerves α, β, γ, δ see text p. 590. × 41.

FIGURE 9. B. lanceolatum. Anterior is up. A portion of the velar plexus, and the nerves of two velar tentacles; *c*, *c*, cells in the epithelial covering remaining attached to the nerves. Cells, or nuclei, in connection with the velar plexus are shown at *n*, *n*. × 123.

FIGURE 10. B. lanceolatum. A portion of the fine plexus of a gonadial pouch. × 343.





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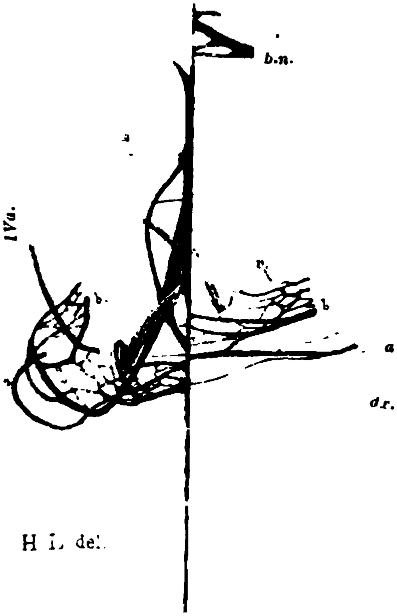

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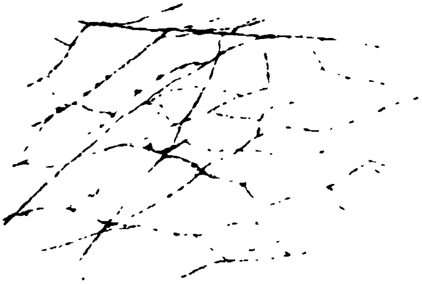

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PLATE 4.

All figures, except 15a, from methylene-blue preparations.

Figure 15. Branchiostoma lanceolatum. The fan-like branches of an ascending visceral ramus of a dorsal nerve, viewed from the interior. The visceral ramus reaches the internal surface of the side muscles at *a*. The branches at *c* and *d* anastomose with those of adjoining ascending visceral rami. The branch at *e* bends toward the exterior. The branches at *b* are bent away from the muscle, i. e. toward the median plane of the animal. × 93.

Figure 15a. Diagram of part of a cross section of an Amphioxus, to show the course of a dorsal nerve and its branches.

Figure 16. B. lanceolatum. View of the inner (median, or atrial) face of the lateral muscles of right side (lower half of figure), and of outer (atrial) surface of the branchial wall (*p. w.*), cut ventrally and turned up (the upper half of figure). To show branches of an ascending visceral ramus passing to the pharynx. Portions of the ligamentum denticulatum are marked *l. d.* The plexus formed by the fan-like branches of an ascending visceral ramus is also shown. × 84.

Figure 17. B. lanceolatum. Similar view to that of Figure 16. Nerves entering the pharynx from the ligamentum denticulatum, and a portion of the plexus of the latter structure. The plexus in the "pocket" portions of the ligament is shown at *pl. d.* The plexus of a primary branchial bar is marked *b. p. 1*, and that of a secondary bar, *b. p. 2*. × 177.

Figure 18. Branchiostoma caribaeum. Anterior is up. A part of the outer plexus of the mouth border, on the *right* side of the body. × 93.

Figure 19. B. lanceolatum. Plexuses on a primary bar, a secondary bar, and a cross-bar of the pharynx, with nerve cells, *c*, *c*, in connection. This is a view of the exterior surface of a part of the pharynx which is near its ventral surface and near the anterior end of the body on the right side; *a*, and *b*, have the same meaning as in the following figure. × 254.

Figure 20. B. lanceolatum. A view of the dorsal border of a portion of the left side of the pharynx from the interior of the latter, showing a nerve plexus. The primary bars are marked *a*, *a*, and the secondary bar, *b*. × 254.

Figure 21. B. lanceolatum. A nerve cell on the external side of a secondary bar of the pharynx. The view is from the right side of the latter, near the anterior end of the body. × 700.

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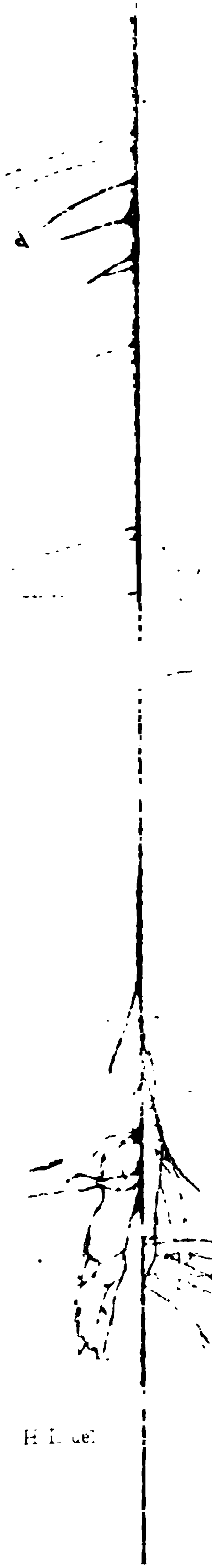

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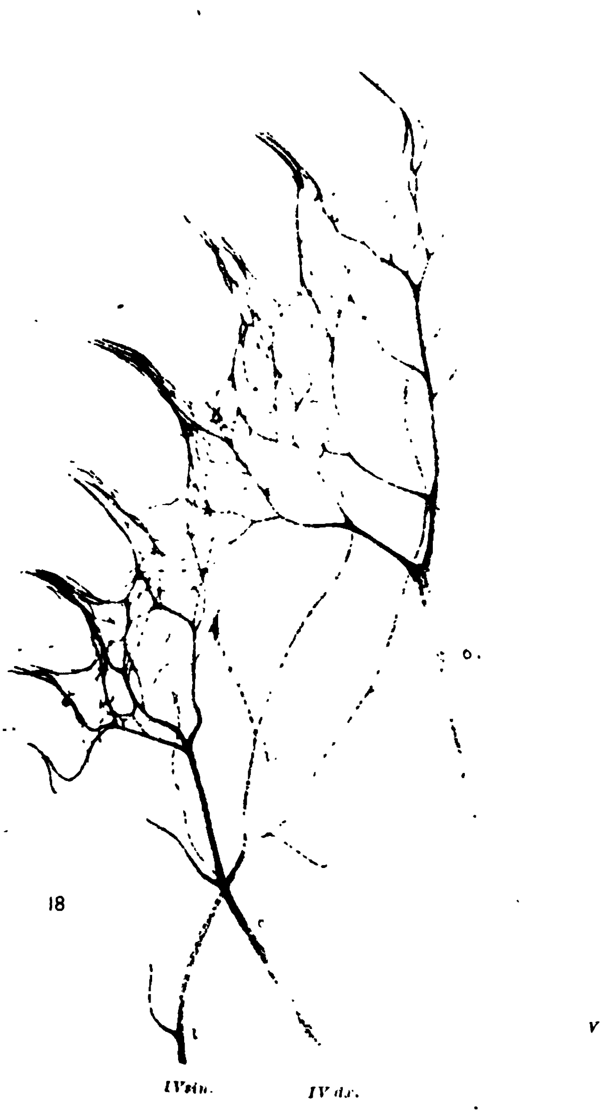

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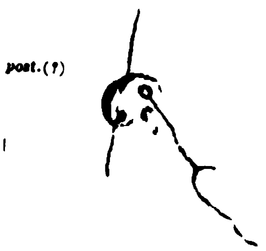

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PLATE 5.

Figure 24 from gold-choride preparation; all others from Golgi preparations.

FIGURE 22. Branchiostoma caribaeum. Transverse section. Nerve fibers entering a dorsal root, in the middle region of the animal. × 238.

FIGURE 23. B. caribaeum. Anterior at the *left* (?). A frontal section. Nerves in the transverse muscles, toward the anterior end of the body, on the right side. × 238.

FIGURE 24. B. caribaeum. A view of the inner surface of skin stripped from the dorsal fin in the region of the 20th myomere from the posterior end of the animal, showing fibers and other structures in connection with a branch of the dorsal ramus of a dorsal nerve. Ordinary epithelial cells are outlined at *e*. Special cells in the epithelial layer are marked *g*, and certain cell-like structures in connection with fibers are shown at *c*, *c*. A star-like place of union of fibers is indicated at *s*. The bases of the epithelial cells lie just exterior to the fibers drawn. × 410.

FIGURE 25. B. lanceolatum. Dorsal aspect of frontal section through the 7th myomere of the right side showing a portion of the corresponding ventral nerve root. × 203.

FIGURE 26. B. lanceolatum. Dorsal aspect of frontal section, next ventral to the preceding one, showing a more ventral portion of the same nerve. × 203.

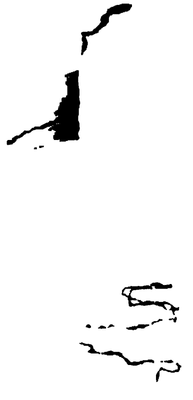
FIGURE 27. B. lanceolatum. Frontal section at the level of the dorsal margin of the notochord, directly ventral to the neural tube. The myomere of the figure is slightly posterior to the middle of the specimen, on the right side. Smooth motor fibers are shown, and a closely associated group of end-plates situated in the side muscles, near the posterior myoseptum. × 141.

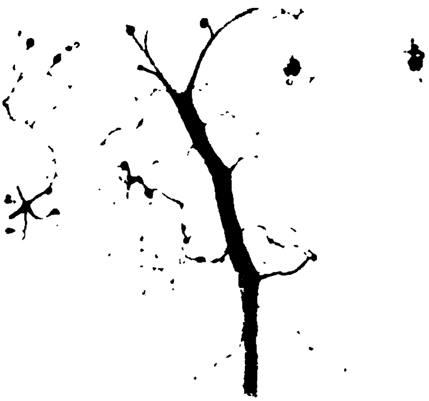
FIGURE 28. B. lanceolatum. Frontal section through the middle of the notochord, showing long motor fibers and end-plates in the side muscles. These muscles are posterior to the middle region of the body. × 141.

FIGURE 29. B. lanceolatum. Dorsal aspect of frontal section, showing motor fibers in the side muscles of the 18th myomere of the right side, in the region of the neural tube just ventral to the place of exit of the dorsal nerve roots. The section is about 46 micra in thickness. × 99.

FIGURE 30. Branchiostoma caribaeum. A nerve cell in the ventral part of the pharynx, in connection with the nerve plexus of that region. The cell lies on the exterior of an endostylar plate. × 226.

LEHI PLATE 5





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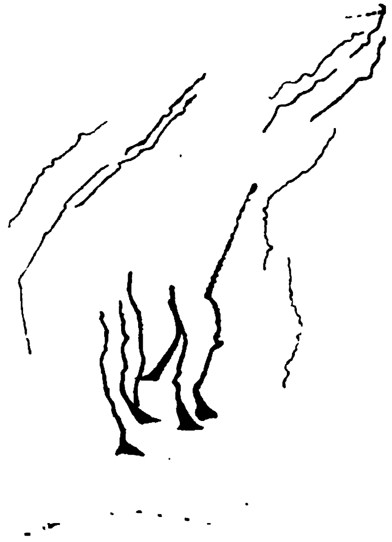

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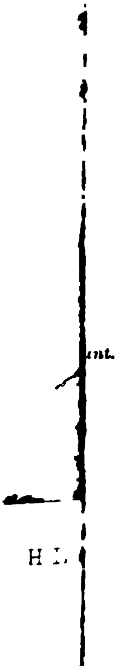

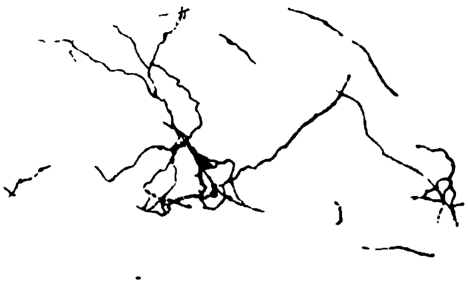

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PLATE 6.

All figures are from methylene-blue preparations.

FIGURE 31. Branchiostoma caribaeum. The same individual as that shown in Figure 33. The nerves of the posterior end, on the right side of the body. × 93.

FIGURE 32. B. lanceolatum. The interior surface of the ventral fin and side muscles bordering upon it, from the region between the atriopore and the anus. Posterior is up. The branches from the ventral rami of dorsal nerves which reach the interior are marked a, a, a. The branch a_1, bends toward the exterior. Part of a membrane, somewhat detached from the side muscles, is indicated at b. The bilateral asymmetry of the muscle segments is illustrated. × 84.

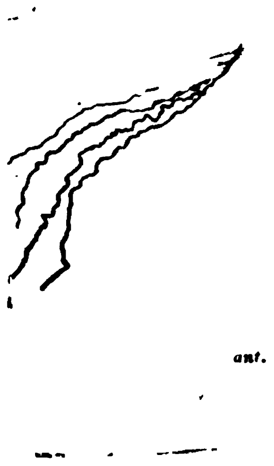
FIGURE 33. B. caribaeum. Nerves of the posterior end of the body, on the left side. The epithelium of the skin is not present. The end of the notochord is bent in the same manner as the terminal ampulla of the neural tube. × 93.

FIGURE 34. B. lanceolatum. Cutaneous plexuses between right dorsal nerves XLVI, XLVII and XLVIII, in the region of the side muscles. The coarser, deeper plexus is marked p_1 (center of figure), the finer, more superficial one p_2 (upper left quarter of figure). A cell-like structure is shown at c, in connection with cutaneous nerve fibers and at a, a nerve passing through myoseptum to inner face of muscle. × 41.

FIGURE 35. B. lanceolatum. Cutaneous plexuses between right dorsal nerves LIII and LIV, and between LV and LVI, in the region of the side muscles. The letters correspond to those of Figure 34. The branch at x joins nerve LVII. × 41.

FIGURE 36. B. lanceolatum. Cutaneous plexuses between right dorsal nerves LIV and LV, in the region of the side muscles. The letters correspond to those of Figure 34. × 84.

PLATE 8



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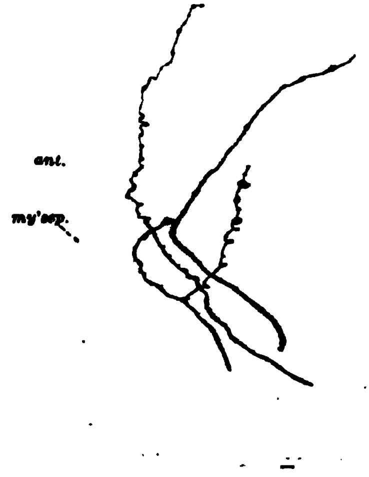

48

50

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
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Vol. 2.

11. **Linton**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

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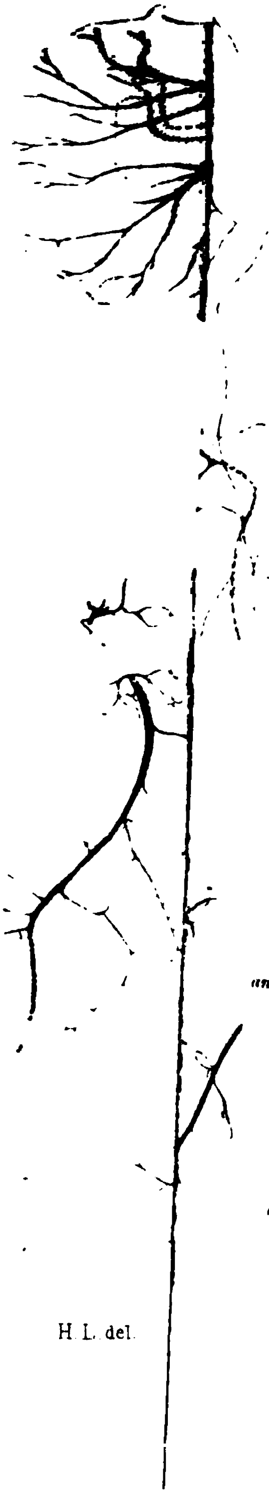

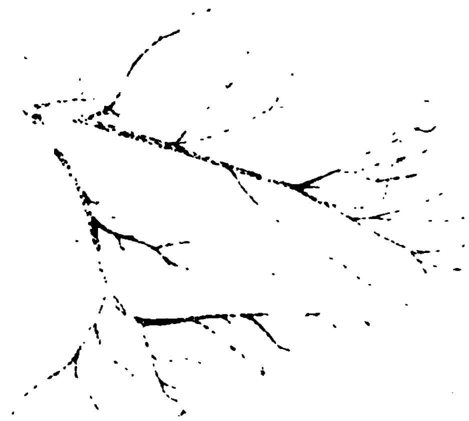

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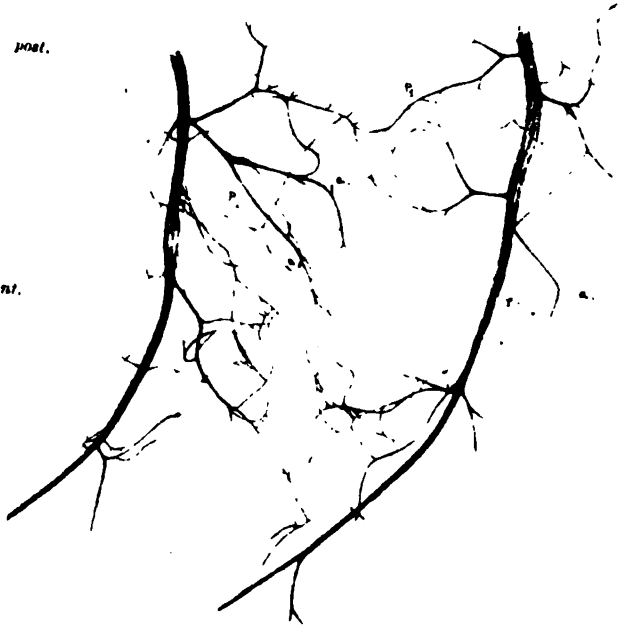

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PLATE 7.

Figures 37–39 and 41 from methylene-blue preparations; figure 40 from Golgi, and figure 42 from gold-chloride (after Ranvier) preparations.

FIGURE 37. Branchiostoma lanceolatum. The exteriorly directed cutaneous branchlets of left dorsal nerve XVI. The epithelium is not present. × 41.

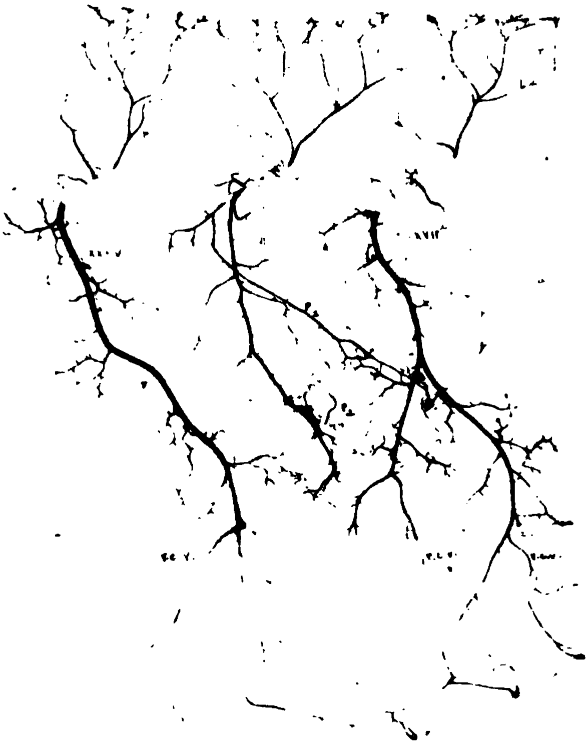
FIGURE 38. B. lanceolatum. Cutaneous plexuses distributed over the side muscles, formed from connecting branches of left nerves XI, and XII. The coarser plexus is marked p_1, and the finer, more superficial plexus, p_2. A visceral nerve branch is indicated at *a*. × 84.

FIGURE 39. B. caribaeum. Cutaneous plexuses between left dorsal nerves XXXV, XXXVI and XXXVII, in the region of the side muscles. The letters correspond to those in preceding figure. × 17.5.

FIGURE 40. B. lanceolatum. Anterior is up. Frontal section. Nerve fibers entering the root of right dorsal nerve XXI. × 238.

FIGURE 41. B. lanceolatum. Dorsal is at the *left*. A plexus formed by the breaking up, for a short distance, of the main stem of the ventral ramus of right dorsal nerve XXVII. × 84.

FIGURE 42. B. caribaeum. A view of the outer surface of skin taken from the region over the side muscles, near the dorsal fin. Special cells are indicated at *g*, and modified cells at *s*. Apparent exudations from the special cells are marked *e*. The exudations lie at higher focus in their distal portions. × 1050.



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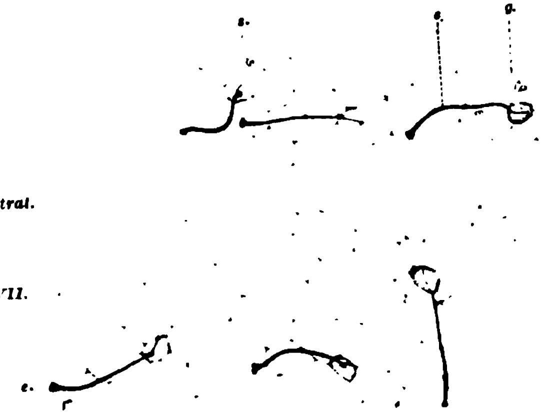

42

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The central nervous system of Tunica nigra.[1]

By

William A. Hilton.

With 11 figures in the text.

Contents.

	Page
Introduction	113
General form of the ganglion	114
The structure of the ganglion	118
The relation of cells to each other	123
Structure of the cells	124
Summary	126

Introduction.

While enjoying the advantages offered by the Bermuda Marine Biological Station, through the kindness of Prof. E. L. Mark, the director, I was able to obtain a large number of specimens of *Tunica nigra.* This form is very abundant on Agar's Island and out on the nearer reefs. Specimens were studied by several methods; some were examined in the fresh condition, while the ganglia of others were dissected out and fixed in various ways for sectioning. Carnoy's fluids were largely used for fixation, and were followed by staining

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1) Contributions from the Bermuda Biological Station for Research, No. 29.

in methylen blue and eosin. Another most serviceable method was to fix with Flemming's fluid and after sectioning to stain with iron- or copper-hematoxylin.

General form of the ganglion.

The ganglion is roughly cylindrical. It is imbedded in the mantle between the oral and atrial siphons. A long neural gland, found on its ventral side (Figs. A, D, E), is more or less closely applied to the nerve center and usually extends its whole length or even farther in the direction of the animal's oral end. It is from this end that a duct of greater or less extent may be followed to such a ciliated funnel as has been described and figured by Julin (1881), Metcalf (1900), and others. The length of this duct from the end of the ganglion to the funnel differs considerably in the various specimens examined. There is also some variation in the position and extent of the duct in the region of the gland. In some individuals this tube, or branches from it, may be traced from the wall of the ganglion itself. That is, it has communication with the nervous tissue similar to that described and figured by Sheldon (1887) in *Clavellina*. This author suggests that the ciliated pit and duct are for the purpose of aerating the brain. Metcalf (1899 and 1900) calls attention to the close relationship between gland and ganglion in tunicates, and in some of his figures shows relations' which might be regarded as histological connections between this duct and the brain.

The chief branches of the ganglion run off from its two ends and take a rather dorsal direction. The usual figures of the ganglion in simple tunicates show only such branches. In some specimens of *T. nigra*, in which the stain was not differential, only these larger,

Fig. A. Drawing of the ganglion and gland of *Tunica nigra*, from a reconstruction. The oral end is up, the ganglion is turned slightly to one side, so that more of the gland, on the ventral side, is seen than would be shown in a strictly lateral view. A duct from the gland shows at the oral end. At various places at the end of the ganglion the stumps of nerve trunks are seen. 17 : 1.

Fig. B. Drawing from a reconstruction of a ganglion of *Tunica nigra*, looking down upon the dorsal surface. Several of the chief nerve trunks are shown, some being a little exaggerated in the drawing, especially the median ones at the lower or atrial end. 17 : 1.

Fig. C. Drawing from a model of a portion of the atrial end of a ganglion in ventral aspect. To facilitate comparison with Fig. B, the atrial end is down, though the shading of the figure is thereby reversed. The cut surface shows a small dorsal median branch cut lengthwise. Other branches are also shown. A ventral view of this part of the ganglion is given to show especially a small lateral fold which extends toward the median plane. 17 : 1.

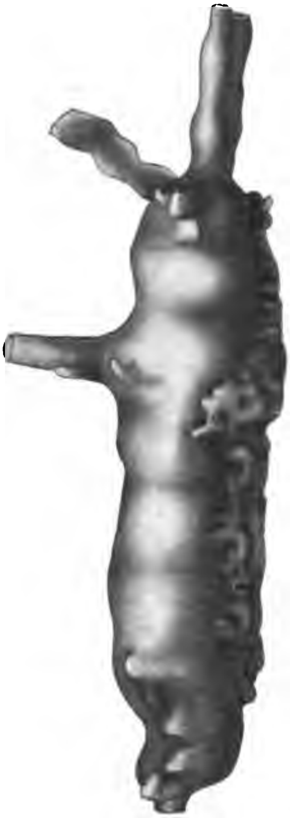

Fig. A.

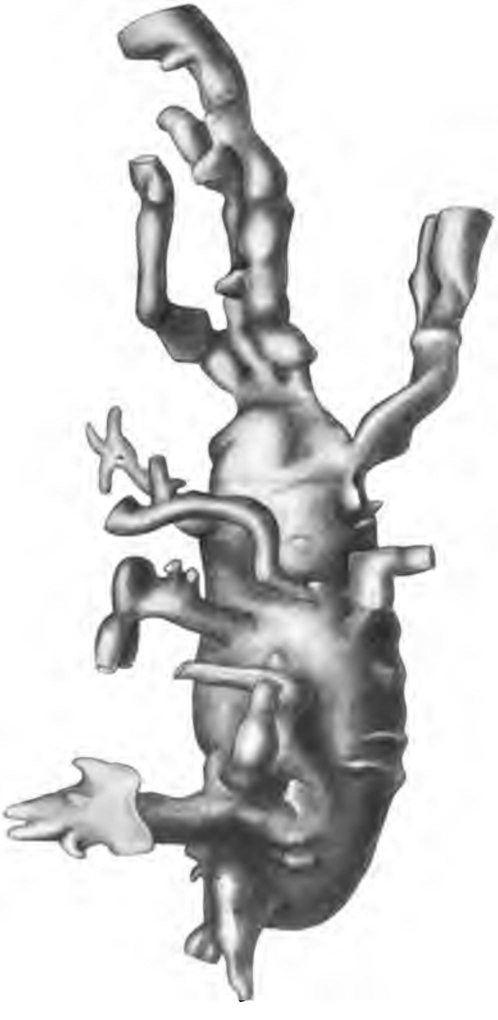

Fig. B.

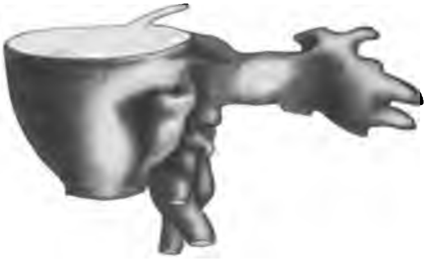

Fig. C.

more or less terminal, trunks were well seen. Fig. A shows a reconstruction from such a specimen; not all of the branches are shown. In preparations stained with hematoxylin and picro-fuchsin, there was a good differentiation of the nerve strands running to the surrounding muscle fibers and connective tissue. In such specimens the nerve trunks could be traced for long distances from the ganglion out to muscles and to the surfaces of the mantle. In favorable specimens of this sort numerous nerves of moderate and small size were also seen coming off from the body of the ganglion.

In the model represented in Fig. B there are three rather large branches shown at the oral end (up in the figure). All of these run up towards this part of the animal between muscle fibers, often crossing from side to side through the thickness of the mantle. They all undoubtedly supply the muscle fibers with which they are closely associated, and also send fibers to the surfaces of the mantle. The central branch, for a time at least, runs along the gland duct. In Fig. A the duct is shown, but no demarcation is shown between the two branches, since they run close together. A more lateral and a more caudal branch, shown in Fig. B, are represented by stubs only in Fig. A. The three terminal branches shown in Fig. B are close together for quite a distance as they leave the ganglion, for a longer distance, in fact, than is to be inferred from the drawing, since their basal portions are represented as one mass, while from sections it is seen that they are distinct down almost as far as the first pair of lateral branches. The larger trunks of the ganglion are often much changed in shape at various parts of their course, as they mould themselves about muscle bands.

In a specimen of a nearly related species, all the branches at the oral end were followed in dissections out to the bases of the oral tentacles. In the specimen modeled, the branches were cut off at that end; but if these branches correspond to those of the other species, then we might expect that they also terminate in the region of the oral tentacles. At least they probably contain both afferent and efferent fibers. The several larger nerves at the atrial end also contain fibers supplying muscles and from their position and general distribution seem to contain afferent fibers also. In the specimen shown in Fig. B there were found in the middle part of the ganglion three large nerves, four slightly smaller ones, and about as many very short and rather minute nerves. Most of these trunk nerves arise from the median plane, but several, such as the small one near the oral end, are more lateral. Just how many very small nerves arise from the ganglion, it is hard to decide, because some nerves are composed of only a few fibers. The two central nerves at the atrial end are somewhat exaggerated in size in the drawing.

There were few cases of anastomosis, at least between the larger trunks. Some of the branches when followed to the periphery were found greatly to change their shpaes owing to the position of muscle fibers; and not infrequently the peripheral part of the nerve becomes swollen to a larger mass than the proximal end.

The end nerves usually do not come off from the terminal portion of the ganglion, but instead, at one side of the tip. Generally the terminal nerves and most of those from the middle of the ganglion come off ventrally. The number and distribution of the larger nerves seem to be fairly constant in different individuals, but the smaller ones vary considerably.

METCALF (1899 and 1900) speaks of the continuation of ganglion cells along the peripheral nerve trunks of tunicates, sometimes in connection with the larger branches, but more often along the rapheal nerve. In none of my sections of this species have I seen nerve cells continued out far from the central mass along the nerves.

As mentioned before, there is considerable variation in the smaller nerve trunks. The rapheal nerve, which METCALF describes and figures, was recognized in a number of specimens, and near it, for a variable distance, a process extending out from the neural gland was considered to be the rapheal duct. The rapheal nerve was small and contained no nerve cells. In some specimens it was short and arose by itself near the atrial end of the ganglion. In another specimen three minute nerves, each with a separate origin, but from the same general region of the ganglion and close to one another, were considered as representing together the rapheal nerve. Some of the strands of these were found supplying muscles.

Aside from these small branches, which were considered to be rapheal nerves, a rather thick mass of nervous matter (a lateral fold) is shown on the ventral side at the atrial end of the ganglion in Fig. C. This body runs out ventrally and ends abruptly, being inclosed in connective tissue. In cross section it looks like the little dorsal projection shown by METCALF (1900) in his figure of *Phallusia* and labeled „ganglionic cord“; or like the figures in his 1899 publication, his Figs. 2, 3, 4 and 5 showing the conditions in

Distaplia, *Amaroecium*, *Ascidia* and *Phallusia*, respectively. I did not find this projection in all the specimens, nor did I find nerve cells in it. Possibly it represents a part of the rapheal nerve cord.

The structure of the ganglion.

The nerve cells are arranged chiefly about the periphery of the ganglion, in cross sections of which they appear as a deeply staining border around the central fibrous core (Figs. D, E). The thickness of this peripheral mass of cells varies considerably in different regions. At various places, where the nerve trunks come off, especially in the region of the larger trunks, the cells of the border become much reduced in number or disappear in large part as the fibers sweep into or from the more central portion of the ganglion (Figs. E, F). Some of the cells are large, others are small, many of the larger ones being located at the outer surface of the ganglion.

Blood is abundant in the gland and is present to some degree also in the peripheral layer of cells in the ganglion.

The central, more fibrous mass of the ganglion is not without cells, but they are widely scattered. Many of these are very small and apparently are not functional nerve cells. The central area contains cells which not only as a whole but as individuals stain less deeply that the peripheral ones (Figs. F, G).

The ganglion is enclosed in a rather dense connective-tissue sheath, which stains pink with fuchsin. In this sheath, and sometimes outside of it, a variable number of scattered nerve cells are found, which seem to be a part of the central system. I found no nerve cells in the gland, but in almost every specimen at some point in the series, gland cells and ganglion cells seemed so completely mingled that it was hard to separate the two areas, a condition such as that described for other tunicates by METCALF (1899 and 1900).

The cells which compose the ganglion are of various sizes from very small ones with almost no cytoplasm to rather large ones 10 or 20 μ in diameter. Small and large sorts are found together both in the center of the ganglion and in the dense masses near the surface. With methylen-blue and eosin stains, the cells appear mostly unipolar, and spherical or pear-shaped; but there are certainly many cells with several processes, although very often one of these is much longer than the others. Very probably the cells which seem to have but one process may have other smaller ones which were not detected. A few cells, especially in the central portion of the ganglion, were found to have several processes of about equal size. Something could be learned about the shape of the neurons and the general direction of their larger branches from methylen-blue preparations obtained in section, but the most perfect cells were obtained from specimens fixed in FLEMMING's fluid and stained

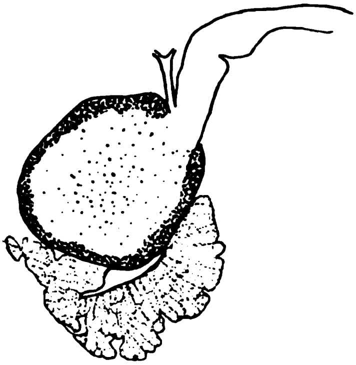

Fig. D.

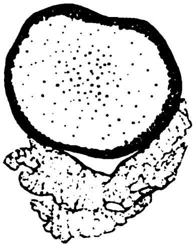

Fig. E.

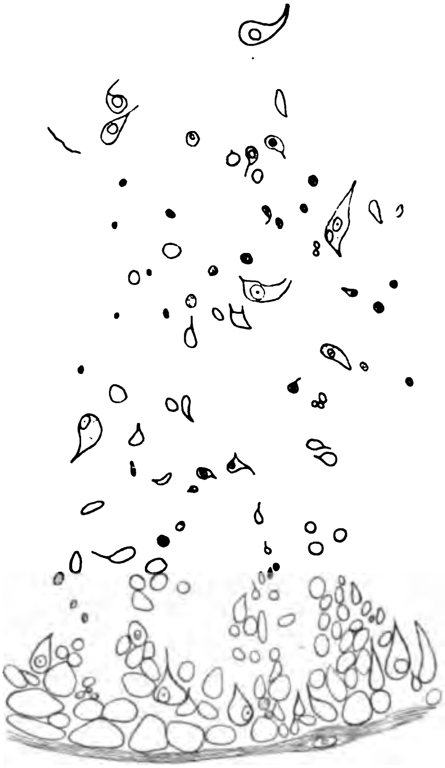

Fig. F.

Fig. D. Drawing of a cross section of the ganglion and gland of *Tunica nigra* passing through the origin of a large and a small nerve. 67 : 1.

Fig. E. Drawing of a cross section through the centre of the ganglion and gland, the gland on the ventral side. 67 : 1.

Fig. F. Camera-lucida sketch from a cross section of a ganglion. The part reproduced extends from the connective-tissue sheath to the center of the ganglion. 450 : 1.

in iron-hematoxylin. A few cells, already mentioned, were found outside the connective-tissue mass, and some were found imbedded in it. The thickness of the outer layer of nerve cells in the ganglion differed considerably in various parts of the same specimen. Some of this difference was due to differences in the plane of sectioning. In places the cells became reduced to a single irregular layer, but in most regions more were seen (Figs. F, G). The average number of cell layers might be said to be three; in some places there were many more than this. Some of the larger elements were often found at the outer edge of the ganglion, many of these were seen to send a large process in towards the center. I am inclined to think that most of the larger ones at the outer edge are of this sort. The branches often run in straight towards the center and are easily followed for some distance. Other cells send their processes to the central area, but not straight in. The direction in which these run is variable; at the same level the process of one cell may be sent obliquely in one direction part way to the center, and near this another cell may send its process obliquely in the opposite direction, but not necessarily at the same angle. Cells of smaller size in the other layers at the periphery were

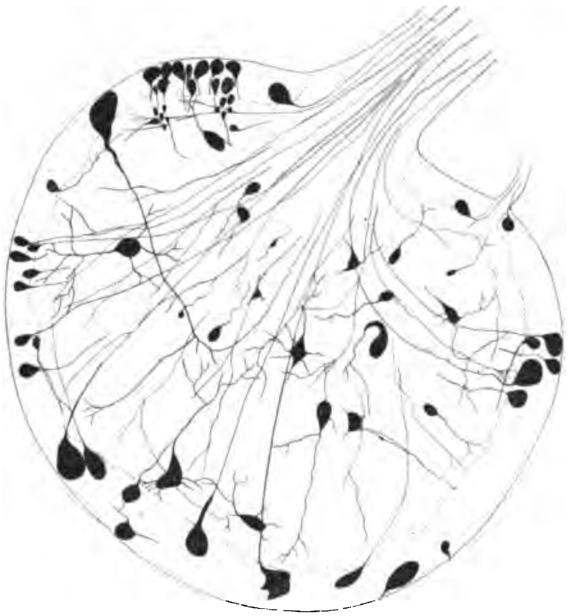

Fig. G. Diagram of the ganglion of *Tunica nigra* as seen in a cross section which passes through a large and a small branch. Drawings of actual cells from various regions are placed in this diagram. Some of the longer processes from nerve cells into the nerve trunk are represented quite diagrammatically, because, so far as could be determined, most peripheral cells send fibers for longer or shorter distances along the axis of the ganglion before entering the nerve trunks. 250:1.

also seen sending branches to various parts of the central fibrous portion of the ganglion (Figs. G, H). A few of the larger more superficial cells of the ganglion were seen to send their processes out into the connective tissue and in some cases apparently beyond this, rather directly into nearby muscle fibers; some of these were undoubtedly the fibers indicated in the model by the smaller median dorsal branches, but it seems probable that some cells as individuals send fibers directly to muscles.

Some of the cells of the peripheral zone could be seen sending processes in among others of this denser mass and in exceptional places a few fibers of this sort were seen inside the general mass of the ganglion. Association cells of large and small size were seen in the midst of the thickened mass. Branches were also seen coming from cells of various levels and running just inside the cortex. Some of these, of course, may have had some efferent fibers as well as association branches. Fibers, apparently for associating various parts of the denser mass at the periphery, were seem to come from cells of all sizes and from all levels, but chiefly from those near the middle of the ganglion. The fibers of these cells at the point of their passage from the peripheral to the deeper layer turn rather abruptly to run for longer or shorter distances along the inner boundary of the peripheral area. Cells of the superficial layers and those farther in towards the center, such as have already been mentioned, usually have one process considerably larger than the others, and in some cases no other processes were seen. Such cells have this portion running in towards the center of the ganglion, sometimes straight, sometimes twisted either near its origin or along its course. Another type of cell was found located at the edge of the ganglion with one large process extending in towards the center of the fibrous mass and with a large branch from this main one running back to another part of the dense cell area (Figs. G, H).

Just inside the denser mass of cells a few scattering ones are often evident. These are much like those of the peripheral thicker mass. The larger nerve branches point in various directions, in some they extend out towards or into the peripheral cell layer; some cells send processes along the inner side of this region as association fibers, possibly in some cases as efferent fibers, while others send their main processes in toward the central part of the ganglion. Some cells of this inner portion of the peripheral mass are very small, often with very little cytoplasm surrounding the nucleus, and exhibit a fine process extending from one end of the cell in towards the central portion of the ganglion and one from the other end extending out towards the periphery. Whether these small cells are functional nerve cells or not, I do not know. At least one of them was found closely applied to the surface of a process from another larger cell (Figs. G, J).

In the more central portions of the ganglion no definite arrangement of neurons was evident. They were irregularly scattered or grouped, sometimes they were very abundant, at other times only a few cells were found. None of them were as large as the largest found at the periphery of the nerve center, and there were many nuclei the processes of whose cells, if they had any, were not found. This was similar to the condition of things found at the periphery of the ganglion. The cells of the central area, because of their isolation, could be studied in some ways better than the others, because all sides of them could be seen. They were found to have a number of processes. Some of the processes from the cells seemed to be confined to the central area, probably for associations in this region, others ran to the edges of the deeper masses at various

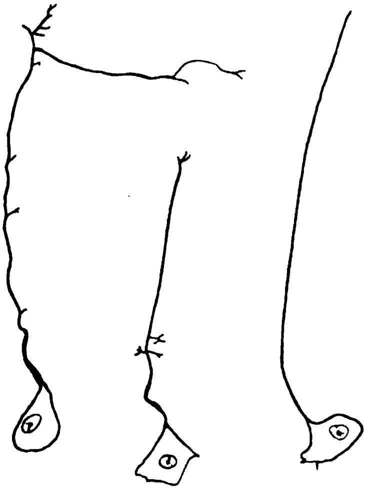

Fig. H.

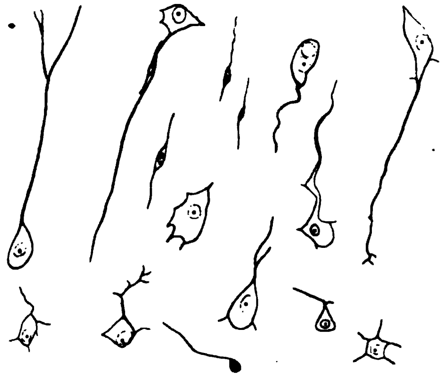

Fig. J.

Fig. H. Four large cells from the peripheral region of the ganglion of *Tunica nigra.* 450 : 1.

Fig. J. Cells from the central region of the ganglion of *Tunica nigra.* 450 : 1.

places, while still others penetrated more or less into the cortex of the ganglion (Figs. G, J, K).

Apparently the central cells are chiefly for association. The fibers from the ones on the surface are undoubtedly to a large degree efferent, the processes for the most part passing through the center of the ganglion on their way to the larger nerve branches. A few large cells, however, at the periphery, as stated, appear to send their fibers out more directly to muscles. Cells at the edge of, as well as just inside, the more compact mass bring various levels of the ganglion into relation with each other. (Fig. G is a somewhat diagrammatic plan of the ganglion as seen in cross section.) The cells shown are in the actual relations as found in different sections, but the efferent and afferent fibers are represented as though prolonged into the nerve trunk, in the plane of a single section, which is a condition not generally realized. Not so many direct communications were found between cells and the nerve trunks, although many individual neurons could be seen to send their strands in towards the center of the ganglion and in some cases direct communications were found. In other words, efferent fibers usually run first to the center of the ganglion and then, after a longer or shorter longitudinal course take exit through the nerve trunks. Receptive cells at the surface of the body, possibly similar to those described by HUNTER (1898a), probably send their processes into the ganglion and may be in some degree related to the central cells. I think it probable that there are not many of these processes as compared with the efferent fibers.

The relations of cells to each other.

In a few rather doubtful cases larger processes of neurons were seen to join with other nerve cells (Fig. K). With specimens stained in iron-hematoxylin after fixation in FLEMMING's fluid, as well as with some of the other material, it was often possible to trace the branches from cortical nerve cells for considerable distances into the depths of the ganglion. In favorable localities these processes were seen to branch and the delicate branches were followed sometimes as far as, and apparently became continuous with, similar delicate fibers of other cells. The minute twigs were often as fine as the fibrillae which were made out in some of the larger neurons, and seemed to be continuous with the fibrillae within the larger processes and cells. In places minute branches from the body of the cell continuous with the fibrillae within it were traced out some distance. These fine processes from cells and from larger fibers were seen in many places and appear to form a delicate network of the more peripheral branches. Such a network of fibrillae was evident in all central portions of the ganglion, but it was only in favorable localities that this meshwork was found to be continuous with the processes of the cells and apparently with those of more than one neuron. This fine network shows to best advantage only in perfectly preserved specimens. The fibrillae may not actually fuse with each other, but the best Zeiss apochromatic oil-immersion lenses gave no evidence of a different condition. No hint was gained by the use of the highest powers as to where the fibrillae of one cell left off and those of an other began. A number of the larger processes of cells could be followed some distance into the ganglion with their numerous apparently anastomosing side branches, until they broke up into minute meshworks and could not be followed farther (Fig. K).

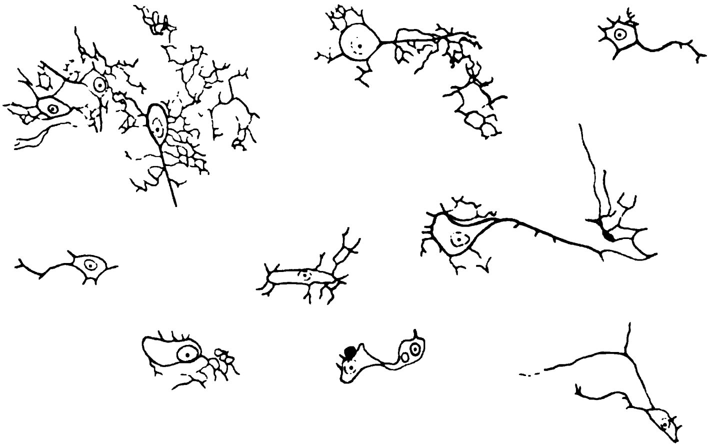

Fig. K. Cells from *Tunica nigra*, chiefly from the central parts of a ganglion. 450 : 1.

Structure of the cells.

Many cells (Fig. L) were found to contain minute fibrillae. These were best shown by hematoxylin methods. Cells stained by erythrosin or eosin and methylen blue gave in varying degrees the appearance of tigroid-like material. In most cells, the deeply staining substance was concentrated into one part of the cytoplasm and not sub-divided into smaller masses; this was especially true of the central cells. In some of the more peripheral neurons the tigroid-like material was not so densely massed and appeared like flakes or dots, but there were few cells of this kind. In many neurons the whole cytoplasm was stained a uniform blue with no indication of darker masses. In cells of medium or large size there wa a large clear nucleus with a large spherical deeply staining nucleolus. In some of the ganglia, there were numerous cells having vacuoles of various sizes, usually only one to a cell; these might be as large as, or even larger than, the nucleus. In a number of specimens these vacuoles were completely filled with a yellowish pigment, which seemed to be best preserved in formalin or Flemming's fluid, but often appeared partially dissolved out even in these fixatives; this gave the appearance of a large vacuole in the cell with a mass of light yellow substance in the center of the clear area (Fig. L).

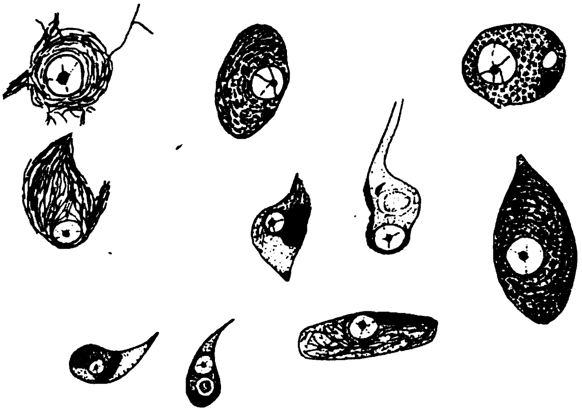

Fig. L. Cells of various sorts from several ganglia of *Tunica nigra.* Fibrillae are shown in two cortical cells, at the left in the figure. Tigroid-like material is shown in the larger cells (which are from the periphery) in the from of numerous evenly distributed, sometimes concentrically arranged, masses or flakes; in the smaller cells (which are from the center), as larger continuous patches. Some of these central cells show in a vacuole a paranuclear mass of yellowish pigment, indicated in the figures as a dotted area surrounded by a clear space. 900:1.

Metcalf (1900) has shown similar "paranuclear" bodies in the nerve and gland cells in tunicates, which he says may be the same as the centrosome structures described in this group. Hunter (1898b) describes in *Cynthia* centrosomes which have about the position in the cells that these pigment bodies do; but I found no indication of centrosomes in *Tunica nigra.*

Summary.

1. The ganglion of *Tunica nigra* possesses more branches than have been described for related forms. Many of these are quite small. The larger nerves seem to contain both afferent and efferent fibers. The smallest branches seem to supply nearby muscles. Some few muscles are evidently supplied rather directly by cells lying at the periphery of the ganglion.

2. The neural gland on the ventral side of the ganglion is in places almost fused with the nerve center, but free nerve cells were not found among the gland cells.

3. The duct from the gland to the ciliated funnel was much like what has been described by Metcalf and many others.

4. Small, somewhat variable strands were recognized in some specimens as rapheal nerves. Another rather thicker mass of nervous substance was found ending abruptly in the connective tissue. This was on the ventral side near the atrial end and was apparently a vestigal structure. Not any of these peripheral nervous structures contained nerve cells.

5 The nerve cells of the ganglion were densely crowded into a cortical layer, the central core being composed chiefly of nerve fibers. There were a few scattered cells located in the central part of this mass of fibers.

6. A large proportion of the cells of the periphery evidently supply muscles. A few cells of the peripheral portion of the ganglion and many of the scattered central cells serve apparently for various sorts of association.

In all parts of the ganglion are cells which are apparently connective rather than nervous in function. These are usually smaller than the nerve cells and have very little cytoplasm.

7. In the central portions of the ganglion is an intricate meshwork of fibrillae, which in places is seen to be continuous from cell to cell and with the fibrillae within the nerve cells.

8. Tigroid-like substance is found in the nerve cells, some-

times as dense masses, sometimes as flakes or dots. Vacuoles, often containing yellowish pigment, are frequently seen in the nerve cells.

9. LOEB (1892) and MAGNUS (1902) have performed experiments to determine the functions of the ganglion in tunicates. MAGNUS concludes that when purely local reactions are obtained from a small group of muscle fibers, the nervous system is not involved at all. The ganglion is the reflex center for the rather simple but general movements of the body. The simple structure with the relatively small number of association cells shows a very simple type of central nervous system.

Bibliography.

BANCROFT, F. W., 1898, The anatomy of Chelyosoma productum, in: Proc. California Acad. Sc. (3), Zool., Vol. 1, p. 309—332, pl. 18.

VAN BENEDEN, E. et C. JULIN, 1884a, Le système nerveux central des Ascidies adultes et ses rapports avec celui des larves urodèles, in: Bull. Acad. Sc. Belgique (3), Vol. 8, p. 15—72, 4 pls.

—, 1884b, Le système nerveux central des Ascidies adultes et ses rapports avec celui des larves urodèles, in: Arch. Biol., Vol. 5, p. 317—367, pls. 16—19.

—, 1885, Recherches sur la morphologie des tuniciers, ibid., Vol. 6, p. 239—476, pls. 7—16.

BROOKS, W. K., 1893. The genus Salpa, in: Mem. biol. Lab. Johns Hopkins Univ., Vol. 2, VII + 396 pp., 57 pls.

CAULLERY, M., 1895, Contributions à l'étude des Ascidies composées, in: Bull. sc. France Belgique (4), Vol. 27, p. 1—158, pls. 1—7.

DELLA VALLE, A., 1882, Recherches sur l'anatomie des Ascidies composées, in: Arch. Ital. Biol., Vol. 2, p. 9—49, pls. 1—3.

FRÖHLICH, A., 1903, Beitrag zur Frage der Bedeutung des Centralganglions bei Ciona intestinalis, in: Arch. ges. Physiol., Vol. 95, p. 609—615.

GANIN, M., 1870, Neue Thatsachen aus der Entwicklungsgeschichte der Ascidien, in: Z. wiss. Zool., Vol. 20, p. 512—518.

HERDMANN, W. A., 1882—1883, On the homology of the neural gland in the Tunicata with the hypophysis cerebri, in: Proc. Roy. Soc. Edinburgh, Vol. 12, p. 145—151.

HJORT, J., 1892, Zum Entwickelungscyclus der zusammengesetzten Ascidien, in: Zool. Anz., Jg. 15, p. 328—332.

— 1893, Same, in: Mitth. zool. Stat. Neapel, Vol. 10.

HUNTER, G. W., 1898a, Notes on the peripheral nervous system of Molgula manhattensis, in: Journ. comp. Neurol., Vol. 8, p. 202—206.

—, 1898b, Notes on the finer structure of the nervous system of Cynthia partita (VERRILL), in: Zool. Bull., Vol. 2, p. 99—115.

JULIN, C., 1881, Recherches sur l'organisation des Ascidies simples. — Sur l'hypophyse et quelques organes qui s'y rattachent, etc. in: Arch. Biol., Vol. 2, p. 59—126, pls. 4—7; p. 211—232, pl. 14.

KOWALEVSKY, A., 1871, Weitere Studien über die Entwicklung der einfachen Ascidien, in: Arch. mikr. Anat., Vol. 7, p. 101—130, tab. 10—13.

KUPFFER, C., 1872, Zur Entwickelung der einfachen Ascidien, ibid., Vol. 8, p. 358—396, tab. 17.

LOEB, J., 1892, Untersuchungen zur physiologischen Morphologie der Thiere, Theil 2, Organbildung und Wachsthum, Würzburg, 1892, 82 pp., 2 pls.

LORLEBERG, O., 1907, Untersuchungen über den feineren Bau des Nervensystems der Ascidien, in: Z. wiss. Zool., Vol. 88, p. 212—248, tab. 14, 15.

MAGNUS, R., 1902, Die Bedeutung des Ganglions bei Ciona intestinalis, in: Mitth. zool. Stat. Neapel, Vol. 15, p. 483—486.

MARTINI, E., 1909, Studien über die Konstanz histologischer Elemente. I. Oikopleura longicauda, in: Z. wiss. Zool., Vol. 92, Hft. 4, p. 563—626, tab. 28—30.

METCALF, M., 1893, The eyes and subneural gland of Salpa, in: W. K. BROOKS, The genus Salpa, Part. 4, p. 307—371, pls. 47—57.

—, 1897, The neural gland in Ascidia atra, in: Zool. Bull., Vol. 1, No. 3, p. 143—146.

—, 1898, The neural gland in Cynthia papillosa, in: Anat. Anz., Vol. 14, p. 467—470.

—, 1899, Some relations between nervous tissue and glandular tissue in Tunicata, in: Biol. Bull., Vol. 1, No. 1, p. 1—6.

—, 1900, Notes on the morphology of Tunicata, in: Zool. Jahrb., Vol. 13, Anat., p. 495—602, tab. 34—40.

RITTER, W. E., 1896, Budding in compound ascidians, basedon studies on Goodsiria and Perophora, in: Journ. Morph., Vol. 12, No. 1, p. 149—238, pls. 12—17.

SALENSKY, 1892, Beiträge zur Embryonalentwicklung der Pyrosomen (Schluß), in: Zool. Jahrb., Vol. 5, Anat., p. 1—98, tab. 1—8.

—, 1893, Morphologische Studien an Tunicaten. I. Ueber das Nervensystem der Larven und Embryonen von Distaplia magnilarva, in: Morphol. Jahrb., Vol. 20, Hft. 1, p. 48—74, tab. 4, 5.

SCHULTZE, L. S., 1899, Die Regeneration des Ganglions von Ciona intestinalis L. und über das Verhältnis der Regeneration und Knospung zur Keimblätterlehre, in: Jena. Z. Naturw., Vol. 33, p. 263—344, tab. 12, 13 (vgl. unter Martini in: Z. wiss. Zool.).

Seeliger, O. und R. Hartmeyer, 1893—1911, Tunicata (Manteltiere), in: Bronn, Klass. Ordn. Thier-Reich, Vol. 3, Supplement, Abt. 1, xi + 1773 pp., 41 tab.

Sheldon, L., 1887, Note on the ciliated pit of ascidians and its relation to the nerve-ganglion and so-called hypophysial gland; and an account of the anatomy of Cynthia rustica (?), in: Quart. Journ. micr. Sc., Vol. 28, p. 131—148, pls. 9, 10.

Ussow, M., 1876, A contribution to the knowledge of the organization of Tunicata, in: Proc. Soc. nat. Hist. Moscow, Vol. 18, part 2, 62 pp., 9 tab. (Russian).

Willey, A., 1892, On the development of the hypophysis in the Ascidians, in: Zool. Anz., Jg. 15, p. 332—334.

—, 1893, Studies on the Protochordata, in: Quart. Journ. micr. Sc., Vol. 35, p. 295—333, tab. 18—20.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pl. Feb., 1907.

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From the Journal of Physiology, Vol. XLVII, No. 6.

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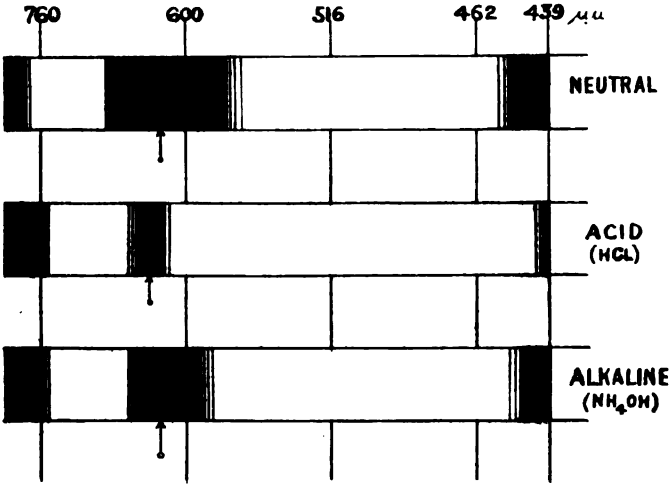
NOTE ON THE PIGMENT OF A BERMUDA NUDIBRANCH, CHROMODORIS ZEBRA HEILPRIN[1].

By W. J. CROZIER.

Chromodoris zebra Heilprin, the largest of the nudibranchs found in Bermuda, has a ground colour of deep blue, present on the general body-surface, the protrusible pharynx, and the foot (excepting its margin and posterior tip). The dorsal and lateral surfaces of the animal are marked with a series of irregular orange streaks. The distribution of these pigments, and their variation in different individuals, is described by Smallwood and by Smallwood and Clark.



I have examined the pigments of this animal in the course of a search for a colouring matter with properties favourable to quantitative studies on the mechanism of its formation in nature. The blue material has several interesting characteristics, which seem worth putting on record. It is readily extracted by weak formalin, alcohol, or acetone, but is insoluble in ether or chloroform. The yellow pigment is not easily dissolved by ordinary solvents.

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[1] Contributions from the Bermuda Biological Station for Research, No. 30.

In solution the colour is not a pure blue, but has a quite perceptible purple tinge. Extracts do not fade noticeably within three months. On the addition of nitric, hydrochloric, sulphuric, acetic or formic acid, the blue colour changes to a very distinct violet-pink[1]. Strong hydroxide of sodium or potassium, or ammonium hydroxide in excess, causes the appearance of a greenish flocculent precipitate, which is soluble in acids.

Absorption spectra of acid, alkaline, and neutral solutions of the same concentration are given in the accompanying figure. The observations were made with a Krüss spectroscope the scale of which had been carefully calibrated. The positions of maximum absorption, as determined by dilute solutions, are indicated by the arrows: they are

neutral	621 $\mu\mu$
acid	629
alkaline	620

If an acid solution of the colouring matter be neutralized, the original blue is restored, but without the violet tinge. This colour change is indefinitely reversible, and suggests the use of the pigment as an indicator.

A number of titrations were made, using N/10 and N/100 hydrochloric acid against ammonium hydroxide, and uniformly gave identical results. On comparing this substance with cochineal, it was found to give the same end-point when using the transition pink-blue. The presence of mineral salts, sugar, alcohol, or formalin, in fairly considerable amounts, exerted no influence on the end-point.

Attempts to obtain the blue material in crystalline form have been unsuccessful.

Similar blue pigments apparently exist in certain other species of *Chromodoris*, for Cockerell describes a blue substance, which is soluble in formalin, responsible for the ground colour of *C. universitatis* and of *C. porterae*, both from the Pacific coast. This blue substance is bleached by potassium hydroxide, and is immediately turned pink by hydrochloric acid.

REFERENCES.

Cockerell, T. D. A. Nature, LXV. p. 79. 1901.

Smallwood, W. M. Proc. Zool. Soc. London. 1910, p. 137. (Contrib. Bermuda Biol. Sta. No. 18.)

Smallwood, W. M., and **Clark**, E. G. Jour. of Morph. XXIII. p. 625. 1912. (Contrib. Bermuda Biol. Sta. No. 26.)

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[1] This is very noticeable when tissues of *Chromodoris* are being killed in Perenyi's fluid, or other acid fixatives.

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9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
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Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16) : 413–455. Apr., 1908.
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14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
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    Separate : 6 pp. Nov., 1909.
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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
FOR RESEARCH.

No. 31.

THE LOCOMOTION OF CHITON.

By G. H. Parker.

CAMBRIDGE, MASS., U. S. A.
April, 1914.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
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No. 81.

THE LOCOMOTION OF CHITON.

By G. H. Parker.

CAMBRIDGE, MASS., U. S. A.
April, 1914.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH, NO. 31.

THE LOCOMOTION OF CHITON.[1]

By G. H. Parker.

The Chiton tuberculatus of the Bermuda Islands affords excellent material for the study of gastropod locomotion. In specimens eight centimeters long, the foot measures about six centimeters in length and two in breadth. Like other species of the genus, this chiton exhibits a retrograde wave-movement on its foot (Vlès, 1907), in that the locomotor waves run from anterior to posterior. As a rule a wave appears at the anterior end of the foot before the preceding one has disappeared at the posterior end; hence the foot may exhibit at any moment one or two waves in progress. Each wave traverses the foot in from twenty to thirty seconds. The waves extend the whole width of the foot and are from five to six millimeters in antero-posterior extent. They represent an area of the foot temporarily lifted from the substrate. This can be easily seen in a chiton creeping on a glass plate in the air. Under such circumstances each wave is marked by an area of air which runs under the foot as the wave begins to form at the anterior end and continues with it until it has disappeared posteriorly. Excepting in the region of the wave, the foot is firmly attached to the substrate; hence at any moment from nine-tenths to four fifths of the foot is fixed and the remainder free. On the free portion of the foot, that is, in the region of the wave, the substance of the foot is moved forward as much as five millimeters for each wave. This, as I have elsewhere pointed out (Parker, 1911, p. 162), is the essential act in this type of locomotion and is characteristic apparently of all creeping gastropods.

Of the two methods found in gastropods for attaching the foot to the substrate, adhesion by mucus, and suction, chiton depends almost exclusively upon suction, but unlike Crepidula fornicata, in which the whole foot acts as a sucker, the foot of chiton sucks locally. If to the foot of an inverted chiton a rigid body with an area five millimeters square is applied, the animal can attach itself to this area with sufficient strength to allow its weight to be lifted. The fact that chiton can thus suck locally with its foot makes it clear how this mollusk can adhere firmly to a perforated sheet of glass from which under like circumstances a crepidula would dislodge itself (Parker, 1911, p. 159).

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[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College. — No. 246.

Chiton tuberculatus exhibits, in addition to its ordinary locomotor movements, certain unusual forms of motion. By swinging the anterior portion of the foot to one side and the posterior portion to the other, the animal can rotate its body with the middle of its foot as a pivot. When the animal is vigorously stimulated unilaterally, as, for instance, by the application of dilute acetic acid to one side of the shell, the whole foot, as seen through glass, can make a purely lateral movement apparently by a single wave that sweeps through the foot sidewise. Finally, chiton can reverse its locomotion and creep backward a few millimeters. The motion is never considerable in amount and is often combined with a lateral movement, but it is unmistakably backward. So far as I know, this is the first instance of backward locomotion to be recorded in gastropods. Because of the infrequency of its occurrence, I was unable to make out the kind of locomotor wave by which it was produced.

REFERENCES.

Parker, G. H.

1911. The Mechanism of Locomotion in Gastropods. Journ. Morph., vol. 22, pp. 155–170.

Vlès, F.

1907. Sur les ondes pedieuses des Mollusques reptateurs. Compt. rend. Acad. Sci., Paris, tome 145, pp. 276–278.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate,* 32 pp., 16 pls. Feb., 1905.
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9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
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Vol. 2.

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13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Moroulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
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19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
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22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
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25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
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3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
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6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
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9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
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19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
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No. 32.

ON THE STRENGTH AND THE VOLUME OF THE WATER CURRENTS PRODUCED BY SPONGES.

By G. H. Parker.

From the Journal of Experimental Zoölogy, Vol. XVI, No. 3.

CAMBRIDGE, MASS., U.S.A.
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ON THE STRENGTH AND THE VOLUME OF THE WATER CURRENTS PRODUCED BY SPONGES[1]

G. H. PARKER

Ever since Grant discovered the currents produced in water by living sponges, this activity has been a matter of interest to the naturalist. The arrangement of the canals within the body of the sponge, the disposition of the motor elements or choanocytes, and the control of currents by means of sphincters have all received much consideration. Very little attention, however, has been given to the currents themselves, their strength and volume.

In a paper published in 1910 in the eighth volume of The Journal of Experimental Zoölogy, I recorded the fact that in Stylotella, a sponge from the coast of North Carolina, the pressure of its currents was equal to that of a column of water from 3.5 to 4 mm. in height. This sponge is of small size and generates only a slight disturbance in the surrounding water as compared with what may be seen in the vicinity of large sponges in the tropical and subtropical seas. Here the water often wells up so abundantly from the sponge as to deform the surface of the sea much as a vigorous spring deforms that of a pool into which it issues. Sponges of this description are frequently met with in the waters about the Bermuda Islands, and, during a recent sojourn at the Bermuda Biological Station, I took the opportunity of measuring the currents from some of these organisms.

The method that I used in making these measurements was the same as that which I had previously employed for Stylotella. A whole sponge or a large portion of one was transported in a tub or bucket to the laboratory and a glass tube of appropriate size was tied securely into one of its oscula. Care was taken to select for this purpose an osculum whose canal system had been uninjured in detaching the sponge from its natural base or, if it had been necessary to break open the canal system anywhere, such breaks were closed by ligatures of soft string. The tube with the sponge attached was then held rigidly in place in the bucket of water and, after five minutes or so, the height of the water in the tube over that in the bucket was measured by means of a millimeter rule attached to the side of the tube. Without disturbing the tube and its attached measure, the canal system of the sponge was then cut open so that the water in this system was in free communication with that in the bucket. The water in the tube immediately fell and, after it had come to a constant level, a reading of its height was again taken. The difference between this reading and that made before the sponge was cut open was assumed to be a measure of the pressure of the water current produced by the sponge.

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[1] Contributions from the Bermuda Biological Station for Research, No. 32.

TABLE 1

Showing pressures in millimeters of seawater exhibited by the currents of seven species of sponges from the Bermuda Islands

NAME OF SPONGE	HEIGHT OF COLUMN OF SEAWATER IN MILLIMETERS FOR FIVE DETERMINATIONS					AVERAGE HEIGHT
Tethya sp. Close to T. seychellensis (E. P. Wright)	2.5	2.0	2.5	2.0	2.0	2.2
Spirastrella sp. Close to S. vagabunda, var. tubulodigitata Dendy	2.5	2.0	2.0	2.5	2.0	2.2
Pachychalina sp.	1.0	2.0	1.5	1.0	1.0	1.3
Spinosella sororia (Duch. et Mich)	3.0	2.5	3.0	3.0	3.0	2.9
Tedania sp.	2.0	2.0	2.5	2.0	2.0	2.1
Stelospongia sp.	2.5	2.0	2.0	2.0	2.0	2.1
Hircinia variabilis F. E. Schulze..	3.0	3.0	2.0	2.5	3.0	2.7

Determinations of the current pressures by the method just described were made in seven species of sponges, and the details of of these measurments are given in table 1. At least five determinations were made for each species. I am under obligations to my friend, Prof. H. V. Wilson for the identification of the sponges upon which these determinations were made.

The surprising feature of the determinations in the table is that they all indicate a very low current pressure, lower even than that of Stylotella. Notwithstanding this general lowness of pressure one of these species, Spinosella sororia, the common Bermuda finger-sponge, produces in the shallow sea a conspicuous deformation of the natural surface of the water, and this deformation can also be observed in specimens that have been transported to the laboratory. As Spinosella was a very satisfactory species to work with, a determination of the volume of water that passed through a single finger of this sponge was attempted. This was made by sinking the tube that had been fastened into the osculum of a finger of Spinosella under the surface of the seawater and thus allowing the water driven by the sponge to escape. The escaping current deformed the surface of the water in the bucket in much the same way as is often seen over a vigorous sponge finger in its natural position in the sea. The rate of the flow of water from the finger was then determined by measuring the velocity of floating particles, such as carmine and so forth, that were carried up the tube by the water current. This proved to be very close to 20 mm. in five seconds. As the diameter of the tube was 17 mm. the finger must have discharged a little over 4.5 cc. of water in five seconds. At this rate the discharge would amount to some 78 liters a day. This finger measured about 10 cm. in length and averaged 4 cm. in diameter; its osculum had a diameter of about 2 cm. Spinosella colonies often consist of as many as twenty such fingers and, assuming that all the fingers work at the same rate as the one upon which the measurements were made, such a colony as a whole would strain in a day about 1575 liters of seawater or over 415 gallons.

Since all the measurements recorded in the table were made upon sponges that had been removed from their natural attachments and transported to the laboratory and since the effect of such treatment is known to retard rather than accelerate the currents, the results therein recorded are believed to be below rather than above the actual working capacity of the several sponges. This opinion is borne out by an observation made on Spirastrella for which I am indebted to Mr. W. J. Crozier and Mr. D. H. Wenrich. A specimen of this sponge was found partly exposed at low water. Although the osculum was out of water, there was still a free flow from it. A cut was made on the side of the sponge at the level of the outer seawater and the sponge was taken to the laboratory. Close measurements showed that the edge of the osculum over which the water lifted by the sponge had been flowing was 4 mm. above that of the sea level, demonstrating that this sponge in the undisturbed condition in which it was found had been overcoming a pressure of 4 mm. of seawater. As the highest pressure that had been obtained from Spirastrella in the laboratory was 2.5 mm., it seems probable that the laboratory determinations for all the species tested are a little below the actual maxima for this form of activity, though, for reasons already given in my former paper on Stylotella, they cannot be much below this maximum.

From these observations it is clear that the currents produced by sponges consist of relatively large volumes of water flowing at low pressure and that currents of this kind are capable of producing such deformations of the surface of the sea as can be observed above large sponges. It would be interesting to ascertain what proportion of the suspended material in the seawater is screened out as the current passes through the sponge, but no observations on this point were made.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

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Vol. 2.

11. **Linton, E.** — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
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Vol. 3.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

No. 38.

THE SENSORY REACTIONS OF HOLOTHURIA SURINAMENSIS LUDWIG.

By W. J. Crozier.

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Page
c) The body surface 253
d) Experiments on mutilated animals 255
e) Discussion 255
2. Continued mechanical stimuli 257
a) Stereotropism; and the righting reaction 257
b) The climbing of vertical walls 259
3. Vibratory mechanical stimuli 260
IV. Light 261
1. Introduction 261
2. Phototropism 261
3. The shadow reflex 265
4. Discussion 273
a) Phototaxis and the shadow reflex 274
b) Theory of photo-reception 274
V. Heat 278
1. Introduction 278
2. Effects of altering the temperature 278
3. Local application of heat 280
4. Discussion 280
VI. Chemical stimuli 281
1. Introduction 281
2. Method; reactions obtained 282
3. Quantitative experiments 284
4. Discussion 288
VII. Summary 291

I. Introduction.

The behavior of those echinoderms which in the adult possess a considerable degree of bilateral symmetry has not received the attention which the morphological peculiarities of these animals would warrant, Of the Holothurioidea, *Thyone briareus* alone has been studied (Pearse, 1908, 1909) with reference to the working possibilities of its nervous system, though there are a number of isolated observations, scattered through the literature, on the habits and reactions of other members of the group.

The present contribution deals with the sensory physiology of an Actinopodous holothurian found in the shore waters of the Bermuda Islands.1) I wish to express my gratitude to Prof. E. L. Mark, who made it possible for me to enjoy the privileges of the Bermuda

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1) This investigation was assisted by a grant from the Humboldt Fund.

Biological Station during the summer of 1913; and to thank, also, Prof. W. M. CHESTER, in charge of the Laboratory during a part of the season, in the absence of Dr. MARK, for many kindnesses. To Mr. D. H. WENRICH I am indebted for assistance during the course of the work.

II. The species studied.

1. Anatomy.

The species chosen for detailed study was *Holothuria surinamensis* LUDWIG (1875). It has been reported from Bermuda by CLARK (1898, 1899, 1901b), and by VERRILL (1906). Figures showing the general proportions of the body are given by CLARK (1901c, tab. 17, fig. 1) and by VERRILL (1906, p. 322, fig. 172). SEMPER'S plate (1868, tab. 28) of *H. coluber* SEMP. gives a more nearly correct idea of the appearance of the animal.[1])

Some 250 individuals were employed in the course of my experiments; smaller numbers of the related forms *H. captiva* LUDW., *H. rathbuni* LAMP., *Stichopus moebii* SEMPER, and *Cucumaria punctata* LUDW. afforded comparative data on certain points.

Though essentially similar in structure to *Thyone* and *Cucumaria* (Dendrochirotae), the genera *Holothuria* and *Stichopus* (Aspidochirotae) present certain anatomical differences which are of importance in comparing the reactions of the two groups. *Holothuria surinamensis* has a vermiform body of approximately constant diameter throughout its length, but narrowing slightly at the anterior end about the mouth and posteriorly about the cloacal aperture. Animals employed in these experiments were usually 10—15 cms in length by 1,5—2,0 cms in diameter, when normally extended. The buccal orifice lies in the center of a pale smooth area, directed somewhat ventrad, and surrounded by a circlet of short peltate tentacles. The number of these tentacles is variable, as in *H. floridana* (EDWARDS, 1908) and other forms. CLARK (1899, p. 121) records 20 as the modal frequency, with a range of 10—21. My observations are in agreement with this statement, and evidence was obtained (see page 249) which indicates that the variation in number of tentacles is in part to be accounted for through regeneration of the anterior end. In specimens about 10 cms long the tentacles are approximately 5,5—6,0 mm in length, 0,5 mm in diameter along the shaft, with peltate discs about 1,5 mm broad. The tentacles have a semicircular valve situated at the base near the ampulla, which when expanded allows the erected organ to be moved about by the muscles of its wall. Immediately behind this circlet of modified tube-feet is a collar, the "brim," with a scalloped edge, which may be so contracted as to close down over the retracted tentacles and the mouth. The ventral surface of the animal is slightly flattened, though not to such an extent as in *H. captiva* or in *Stichopus;* on it occur the irregularly scattered pedicels, about 10—16 of them per square centimeter. The pedicels are about 2,5 mm long when normally extended. On the dorsal and lateral surfaces are slender tapering papillae, modified pedicels, which bear no sucking-discs; they are 2—3 mm long, usually, and are not so numerous, relatively, as the pedicels. The papillae are frequently borne on blunt tubercles. Occasionally papillae with divided or duplicate tips were found. The cloaca terminates in a circular opening, which is rhythmically opened and closed by special muscles; the pumping action of the cloaca — accomplished by muscle strands running to the body-wall and by an anal sphincter, and assisted by the activity of the general body musculature and the muscles of the respiratory trees (Henri, 1903d) — forces water in and out of the respiratory trees. Surrounding the cloacal aperture is a brim, which bears a distinct ring of papillae. The color of *H. surinamensis* is a general brown, varying from a yellowish tint to olive and occasionally to almost black; the ventral surface is much lighter than the dorsal, while the tentacles, oral surface, edge of the collar, pedicels and tips of the papillae, are yellow. Darker patches are sometimes present on the upper surface, especially about the bases of papillae.

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[1]) CLARK (1899, p. 121) finds that Bermuda specimens differ slightly from LUDWIG'S original description of *H. surinamensis* from Surinam, in minor features of the calcareous deposits of the skin and "lantern". While these differences are not considered to be of specific rank, they are pointed out also in specimens from Jamaica and Porto Rico (CLARK, 1901c, p. 359).

Of the internal arrangements, which conform to the description of conditions in other well known species of *Holothuria,* attention need only be called to the five radial bands of longitudinal muscles and the interradial muscle strands perpendicular to them. Three of the longitudinal bands constitute the ventral "trivium", the two dorsal ones forming the "bivium". The walls of the podia are also contractile. Special retractor muscles, such as control the extension of tentacles in the *Cucumariidae,* are not present in *Holothuria.* Histologically the muscles are composed of unstriated spindle cells, which show a faint longitudinal fibrillation (HAMANN, 1883, GEROULD, 1896). Their physiology (in *Holothuria*) has to some extent been studied by BIEDERMANN (1889). From the fact that the musculature of *Holothuria* is made up of nonstriated cells, it must not however be inferred that it is physiologically comparable to the nonstriated contractile tissue of vertebrates. The experiments of v. UEXKÜLL (1896) and others show that, in general, the skeletal muscles of invertebrates (as contrasted with the visceral musculature) contract rapidly in response to a variety of stimuli, as do the skeletal muscles of vertebrates.

The manner in which the muscles are concerned in the body movements of *Holothuria* has been discussed at length by HÉROUARD (1889, pp. 592 et seq.), and in many text books. The essential point is that the muscles work upon the incompressible fluids of the body cavity. After being contracted, the body is re-extended by the relaxation of the musculature and the pumping in of water.

The sense organs and the conducting portion of the nervous system will be considered in the discussion of the experimental results. *H. surinamensis* is not a "cotton-spinner." The Cuvierian organs are found in only a few individuals, and even then but poorly developed. *H. captiva*, however, discharges its sticky "cotton" on the slightest provocation; CLARK (1901c, p. 257) notes that in this species the Cuvierian organs may amount to as much as one tenth of the total bulk of the individual.

2. Habitat and activities.

The life of *Holothuria* in its normal habitat was studied with the purpose of obtaining a proper background on which to view the results of laboratory experiments.

a) Habitat. According to CARPENTER (quoted by CLARK, 1889, p. 122) *H. surinamensis* "can be picked up almost anywhere along the reef protected shore...among broken rocks", and VERRILL (1906, p. 278) states that it also occurs on the reefs. While specimens were found in such situations, they were much more plentiful, near the Agar's Island Station, in certain sheltered shallow bays where the bottom, formed of a considerable thickness of rather loose mud, was covered with masses of matted seaweeds[1]) and sponges. The holothurians occurred buried in the mud about the stems of water plants and the roots of mangrove trees, under stones at or just below low waterline, and in small cavities in the aeolian limestone of the shore. In some restricted areas where the holothurians were most frequently collected, the bottom was exposed for an hour or more at each low tide; during this time the animals burrowed deeply. Occasionally they were observed partially exposed, the anterior end being buried and the respiratory cloacal end appearing above the mud. Early in the morning, before the sun had risen, the holothurians were found lying freely exposed upon the bottom. They slowly disappeared into the silt as the day-light became brighter. In the evening, however, they were not found at the surface — at least up to twelve o'clock — even on dark nights. While I have not sufficient evidence to warrant the statement that this emergence from the mud is a daily occurrence, it would seem that *H. surinamensis*, like the species described by DALYELL (1851, Vol. 1, pp. 49 et seq.) and the holothurians observed by many later workers (cf. SEMPER, 1868, p. 201), is essentially a nocturnal animal. Perhaps some special combination of tidal and light conditions is necessary to bring the animals to the surface. When first collected the animals were covered by a thin layer of fine silt, apparently held by some mucous secretion, which adhered tightly but could be peeled off in flakes. This covering did not extend to the tentacles, pedicels, or tips of the papillae — possibly because these parts exercise respiratory and sensory functions. The muddy investment gives the holothurians the exact color of their surroundings, especially when living among rocks.

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[1]) Most prominent among the water plants were species of Penicillus,

b) Temperature range. The range of temperature to which *Holothuria* is naturally subjected is of considerable magnitude. Surface water in the Bermudas has in summer an average temperature of 31°± (PARKER, 1908, p. 438, and my own observations); it is higher in more open areas: 35°± (81—84° F, MARK, 1913). A series of temperature readings at different hours of the day and night indicated that the temperature range, in places from which holothurians were taken at the time the observations were made, was at least 12° — extending from 20° to 31°. The chief factor in the production of this temperature variation is the state

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Halimeda, Laurencia, Acetabulum, Polysiphonia, some filamentous forms, and Zostera. I am indebted to Mr. F. S. COLLINS for these identifications.

of the tide, which at low water may expose the bottom to the direct action of the sun; the matter is further complicated by the fact that, at the shore line, cool water seeping from the porous limestone tends to lower the temperature in certain spots.

c) Feeding. Food ingestion is accomplished by *Holothuria* with the aid of the tentacles, which, like the buccal pedicels of spatangoids and ophiuroids (GRAVE, 1902, HORNYOLD, 1909), shovel loose bottom material into the mouth (cf. SEMPER, 1868, p. 103). The large doubly flexed intestine is always full of sand, which contains numbers of Foraminifera (*Orbiculina*, etc.), and other minute forms, small mollusks and bits of shell. After the abstraction of nutritive materials from the ingested sand, the inert residue is passed out through the cloaca in a state of fine sudivision.[1]) This is a matter of some geological importance, as there is good ground for believing that through their mode of feeding holothurians play an important role in the secondary decomposition of coral formations, as has been brought out especially by the work of GARDINER (1903, pp. 333—341).

In the *Cucumariidae* there is a characteristic system of feeding reflexes, which involves (DOHRN, 1875, GRAVE, 1902, PEARSE, 1908) the waving of the tentacles through the water or over the bottom, and their consecutive cleaning by being wiped off in the mouth; certain tentacles, in *Thyone* the two short ventral ones, are used more frequently than the others. No preferential use of particular tentacles was noticeable in *Holothuria surinamensis*.

d) Respiratory movements. In laboratory aquaria the respiratory movements of the cloacal aperture occurred with considerable regularity, at the rate of 1 min. 10 secs. for ten pulsations at 27°. These movements are essentially similar to those carried out by *Thyone* (PEARSE, 1908, p. 271), *Caudina* (GEROULD, 1898) and other holothurians, and need not be described in detail. The rhythmic opening and closing of the cloacal brim is interrupted at irregular intervals, either by a more powerful inspiration, or by a complete closure of the orifice, which may persist for some minutes; such interruptions occurred usually in company with spontaneous contractions of the anterior end and retraction of the tentacles. During "respiration" the time occupied by the open phase exceeds that of the period of closure. That the mechanism concerned with rhythmic movement of the cloaca is strictly local is shown by the following experiment:

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[1]) The literature dealing with the processes of nutrition in holothurians is summarized by JORDAN, 1913, Vol. 1, p. 257—266.

Exp. 92,2.

July 28. 5,00 P.M. Posterior ends 5—6 mm long removed from five animals.

9,00 P.M. Edges of wound turned in, but closure not complete. Cloacal end was opening and closing rhythmically; time for ten pulsations = 1 min. 9 secs. 27°.

July 29. 9,30 P.M. Rhythm of anal pieces continued; time for ten pulsations = 1 min. 22 secs. 27,5°.

The regular movement interrupted at about every seventh closure by one of greater vigor and completeness, as in the intact animal.

July 30. 2,30 P.M. Rhythmic movements continued, though less vigorous; time for ten pulsations = 1 min. 50 secs. 27°.

Aug. 1. 9,00 A.M. Pieces dead and decomposing.

Rhythmic movement of the cloaca was maintained for about two days by small pieces cut from the posterior end, with a rate and vigor at first normal but gradually falling off as the pieces became exhausted. There was, however, a notable absence of the periods of cessation exhibited in these movements by the intact animal. This points to a possible controlling action of the nervous system, or to the continuous stimulating effect of poor oxidation conditions on the excised pieces. The latter explanation is less probable. Edwards (1909, p. 215) notes that rhythmic movements of the cloaca appear in a very early stage of the free larva of *H. floridana*, before the respiratory trees are developed. Hence there would seem to be located here an independent mechanism for rhythmic contraction.

e) Locomotion. With *H. surinamensis* and *rathbuni* the force of attachment of the tube feet is never very great, and they may readily be loosened from rocks or the bottom of an aquarium. Indeed, a freeing from attachment to the substratum is one type of reaction to general mechanical stimulation. *H. captiva* and *Stichopus*, on the other hand, can rarely be detached without tearing numbers of the pedicel-stalks. This difference in behavior is correlated with the nature of the habitats of these species; *H. surinamensis* generally lives in relatively soft mud, as does *rathbuni* (though it was found in sand also), while *Stichopus* occurs freely exposed on harder bottoms and *H. captiva* was found, even in very young stages

(0,6 cm long), on the under sides of large stones in situations where there was often considerable wave action.

On a solid surface *Holothuria* progresses by the combined action of pedicels, buccal tentacles, and a peristaltic activity of the body musculature (cf. Hérouard, 1889, p. 539). The last factor is not always evident, and the first one is relatively unimportant, except in the larva (Edwards, 1909, p. 217). My observations on the rôle of the pedicels agree with those of Pearse (1908, p. 265), in that in neither *Thyone* nor *Holothuria* is there any pushing action of the pedicels such as Jennings (1907, p. 99) described in the horizontal locomotion of *Asterias forreri*. The body is never lifted clear of the substratum. The tube feet become much more readily attached to a rough surface, such as that presented by a stone, than they do to the smooth surface of a glass plate. Progression motions always begin at the anterior end, with the tentacles extended; the most anterior pedicels contract first, and a sort of wave of contraction passes down the array of tube feet. In *H. captiva* there is some lack of coördination observable in this process, as the most posterior pedicels are frequently much stretched, and pulled loose, by the forward extension of the anterior part of the body. The rate of locomotion on a horizontal smooth surface is of the order of magnitude of one centimeter in four minutes, in the absence of directive stimuli. Under certain conditions the body may, in crawling, become twisted into a more or less complete spiral on the long axis; such twisting begins at the anterior end, which in this process is enormously elongated as compared with its ordinary appearance. Neither is the animal rigidly straight, for it may become bent at any level, and even completely doubled on itself. Progressive waves of local contraction and relaxation of the body musculature have been mentioned as 'peristaltic' waves; such waves were observed to begin at any level, and to move anteriorly or posteriorly with about equal frequencies. They are rather slow, the most rapid ones traveling at a rate of one centimeter in four to ten seconds, and their principle function in locomotion is the extension of the anterior end of the animal.

Cole's (1913a) statistical study of the movements of the starfish *Asterias forbesi* in the absence of directive stimuli has brought out the fact that the part of the animal most frequently in advance is the region in closest proximity to the madreporite. This 'physiological anterior', as Cole points out, corresponds to the morphological anterior of spatangoids, and the interesting possibility suggests itself that the intensity of physiological polarization[1]) in echinoderms is correlated with the degree of morphological differentiation about a longitudinal axis. With the holothurians, in which this sort of anatomical arrangement is most obvious, homologies are made difficult by reason of the assumption, in these forms, of a horizontal axis morphologically perpendicular to that of the Asteroids and Echinoids. *Thyone briareus*, the only pedate species which has thus far been studied, apparently moves with any part of the body to the front (PEARSE, 1908, p. 278). *Holothuria*, however, affords considerable support to COLE's idea, for the three species of this genus which I have studied were never observed to crawl in any other way than with the anterior end in advance, and the same statement applies to *Stichopus*. The locomotion of *H. surinamensis* was carefully studied with respect to this point. Backward crawling could not be secured by seemingly appropriate stimulation experiments; the direction of *H. surinamensis*' progression is as definitely fixed as is that of gasteropod mollusks (cf. PARKER, 1911). To test the possibility of reversed crawling, a number of animals were placed in horizontal glass tubes of such a diameter as to allow only a little space between the wall of the tube and the animal's body. Irrespective of the direction of the incident light, movement was always in a forward direction. In tubes with one end sealed, holothurians would remain with the tentacles closely pressed against the sealed end until death ensued (in 12—24 hours) through the absence of adequate oxidation and the toxic action of excretory products. A few individuals managed to escape from tubes having one end closed, but this was accomplished by so twisting the body as to bring the anterior end toward the opening of the tube. It is probably in this manner that the holothurians adjust themselves in narrow holes in rocks with the tentacular end outward. Burrowing is accomplished, as in *Synapta* (CLARK, 1901a), *Caudina* (GEROULD, 1896), and other holothurians (SEMPER, 1868), in a slanting direction, with the anterior end directed downward. Animals from which the anterior end, including the nerve ring and associated structures, had been removed by a transverse cut, likewise consistently moved with the anterior end to the front, as, for example, in the following experiment:

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1) The term polarization here means merely antero-posterior differentiation.

Exp. 33,1.

July 3. 4,05 P.M. Three animals had nerve ring, etc., removed.
5,15 P.M. Tube feet expanded, and waving about in normal fashion.
9,00 P.M. "Decapitated" pieces gave a well-defined orienting reaction to light from an oil lamp, moving away from the light (see page 261) anterior end foremost. Quickly exhausted after 10 minutes stimulation.

The phenomena of locomotion in *Holothuria* point to the existence in this animal of a strongly developed physiological polarity, analogous to that seen more vaguely in *Asterias* and in spatangoids. The reason why this polarity is not so clear in *Thyone* is to be found, I believe, in the fact that this form bears tube-feet scattered over its whole surface, whereas those of *Holothuria* are strictly confined to its "ventral" side, or trivium.

f) Auto-evisceration. The curious habit of casting out the viscera in response to intense irritation, or when the conditions of existence have become particularly unfavorable, has long been remarked in holothurians (DALYELL, 1851, and many later workers). It is not confined to this group, but is exhibited also by crinoids (*Antedon*; DENDY, 1886) and starfishes (*Cribrella*; COLGAN, 1912). PEARSE (1909) studied visceral autotomy in *Thyone* with special reference to the rôle played by the nervous system. In agreement with the views of CUÉNOT (1891) and of CLARK (1901), he regarded autotomy as a result of pathological conditions, probably operating through a reflex system of the sort known to exist among arthropods. There would seem to be several distinct grades of autotomy practiced by holothurians: *Synapta* (PEARSE, 1909) pinches off fragments from the posterior end, and the reaction depends on the presence of the nerve ring; *Thyone* (PEARSE, 1909) ejects the viscera through a rupture in the body-wall near the calcareous ring; holothurians possessing Cuvierian organs cast them out through the cloaca, and may or may not accompany them with more or less of the intestine, depending on the species and the exciting circumstances. To these categories of autotomy must be added the process of self-division (DALYELL, 1851, see also p. 246) employed (?) as a method of reproduction.

It was mentioned in the section on anatomy that *H. surinamensis* has the Cuvierian organs poorly developed. The discharge of "cotton" is therefore not a feature of the animal's behavior; but under certain conditions the gut, respiratory trees, etc. were cast out through the cloacal aperture, or even through the mouth (though this was unusual), or (if the animals were in very poor tone) through holes in the body-wall made by a process of local degeneration very similar to that which occurred when death was brought about by heating, and not marked by any great muscular activity. The time required for the completion of the act of autotomy varied greatly with the experimental conditions, occupying from a few minutes to an hour or more.

I have been unable to discover the exact circumstances which determine the casting out of the internal apparatus; once started, however, the principal factor involved in forcing out the viscera is a slow muscular peristalsis working on the pressure of the fluid, in the body-cavity. When an animal was picked up and handled, strong muscular contraction forced water out through the cloaca, and as the aperture was quickly closed considerable internal pressure was developed. If now a cut was made in the body-wall, or if the animal was divided transversely by a sharp clip with scissors, part of the viscera might be suddenly extruded; but this is not to be regarded as an act of autotomy; evisceration sometimes followed, hours after the operation, though mutilation did not invariably lead to this result (cf. TORELLE, 1909).

Exp. 72,1.

July 14. Twenty-three specimens had part of body removed — anterior end, 13 cases; posterior end, 10 cases. Of these, 7 autotomised part or all of the gut after periods ranging from 10 min. to 14,5 hrs. The remainder regenerated without autotomy. All the animals were kept under practically identical conditions, in aquaria with normal animals which did not autotomise.

The only stimulus, or complex of stimuli, that invariably produced autotomy was the stagnation of the sea water in which the animals were living; but individuals kept in aquaria under constantly running water occasionally eviscerated also, after they had been in the laboratory for some time. With the idea that depletion of the available oxygen or the accumulation of some excretory product was responsible for autotomy in "stale" sea water, experiments were made with boiled sea water1), and with water to which carbon

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1) These experiments were carried out using sea water which had been boiled, the loss by evaporation made up with boiled rain water (volumes corrected for temperature expansion), and allowed to cool in full sealed jars;

dioxide, uric acid, or urea[1]) had been added; low concentrations of tannic acid were also used. These experiments gave no definite results, as autotomy was produced with some individuals and not with others; and even in the first instances autotomy was observed only after the lapse of such an interval (10—12 hours) that sea water, originally normal, might have produced the same effect. That some pathological state is induced by the stale water is strongly indicated by the fact that animals kept over night in a small volume of seawater were frequently observed to eviscerate after being transferred to fresh sea water; and further, that after the holothurians in an aquarium had begun to eviscerate, aëration of the water by splashing did not put a stop to the process.

Chemical irritation of a different sort was found to initiate autotomy in a majority of cases. It is almost impossible to anaesthetise *Holothuria* and keep it intact. Carbon dioxide was the most successful anaesthetic employed; the use of chloral hydrate, magnesium sulphate, chloretone, cocaine, or urethane did not give good results. Immerson in fresh water led to partial evisceration in four out of ten experiments. Dropping the animals into formalin (5 per cent, or stronger) or into 70 per cent alcohol, invariably produced more or less complete autotomy when some of the irritating material had been drawn into the cloaca in the course of a respiratory movement.

The integrity of the nervous system is not essential for the act of autotomy, though it is more successfully carried out by the intact animal. The following experiment will illustrate this point:

Exp. 85,2.

2. July 21. 10 animals had 5 — 7 mm. of the anterior end, including the ring structures, removed by a rapid cut; 8 of these showed part of the respiratory trees extruded through the cut immediately after the operation; 2 individuals expelled the gut through the new anterior end within five minutes after the operation. No further autotomy observed in this lot; kept until July 25th.

The only conclusion which seems justified from these experiments is that visceral autotomy is the result of a pathological con-

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when cool, the holothurians were introduced and the jar quickly resealed. The resistance to lack of oxygen shown by Holothuria is paralleled by that of many other marine animals (MOORE, 1913).

[1] FOSSE (1913) has identified urea in echinoderms and their excretory products.

dition of the whole organism, and that it is not absolutely dependent on the presence of the nerve ring. The fact that intact individuals were more successful is explicable on the basis of a great loss of muscular tone suffered by mutilation. The rôle of the so-called central nervous system in autotomy as seen in various holothurians is analogous to that which this organ plays in the regeneration of diverse forms — in some (*Synapta*) it constitutes part of a necessary complex, while in others (*Holothuria*, and possibly *Thyone*) it does not.

g) Regeneration.

α) In nature. Approximately ten percent of the animals observed (*H. surinamensis* only) were found to have anterior or posterior ends much lighter in color than the rest of the body. Posterior ends of this type were more frequently encountered than anterior ends — twenty of the former and twelve of the latter being noted among three hundred specimens. No case was observed in which both anterior and posterior ends were of this kind. The size of the parts referred to is seen in Table I. Such terminal regions were readily recognized by their pale yellow pigmentation, less than normal diameter, and (especially in the case of the posterior ends) their low degree of contractility when stimulated. Tentacles on these ends were thin, short and very slightly pigmented. The possibility suggested itself that these lightly colored parts are formed by regeneration, subsequent either to autotomous bisection, or to injury by such enemies as fishes and the large blue crabs (*Callinectes*) which abound in localities where *H. surinamensis* was found. The latter explanation seems improbable, for the integument of *Holothuria*, though relatively thin, is very tough and not easily cut. There is no evidence that *Holothuria* has enemies of this kind. Moreover, the fact that other holothurians (DALYELL, 1851; Vol. I, p. 74 et seq., tab. 14; and later workers) are known to show self-division, coupled with the remarkable powers of regeneration exhibited by other genera, makes the first suggestion the more probable. TORELLE (1909) found that mutilated fragments of the Aspidochirota studied in her experiments (including three species of *Holothuria*) were extremely tenacious of live, but did not readily regenerate. Experiments shortly to be discussed showed that anterior and posterior ends exactly similar to those observed under natural conditions were formed in a short time after the removal of the appropriate part. While I have not seen a case in which self-division was in progress, it is nevertheless entirely possible that this process is a normal mode of reproduction in *H. surinamensis*.

Table I.

Size of the regenerated(?) terminal regions — *H. surinamensis*.

Total length extended cms	Length of anterior regions cms	Length of posterior regions cms
6,7	—	1,2
10,0	1,4	—
10,0	—	1,0
12,0	1,0	—
12,3	2,0	—
14,6	—	0,8
17,0	2,3	—
17,5	2,4	—
18,0	1,2	—

β) Experimental. In connection with experiments on the nervous system, operations were performed on *Holothuria* which incidently brought to light some interesting phenomena of regeneration. Since the observations of Dalyell (1840, 1847) it has been known that some holothurians are able to make good the removal of parts of the body, but Torelle's (1909) work indicates that in the genus *Holothuria* this power is limited.

When the body wall of *Holothuria* was cut, there followed a set of activities making for the closure of the wound and the reparation of the lost part. Precisely as in the case of coelenterates and annelids (cf. Rand, 1909), a prominent feature in the early steps of wound reparation is the inbending of the cut edges; while there is some question as to the extent to which various factors, such as muscle contraction, amoeboid activity of tissue cells, etc., are concerned in this process, experiments on *Holothuria* demonstrated that both muscular activity and the slow movement or multiplication of superficial tissue cells is involved. In the first experiment thin fragments of the outer body layer were removed, without cutting into the body-cavity, and the manner in which the surface thus exposed was covered over was watched.

Exp. 94,2.

July 29. 11,30 A.M. Thin pieces of the outer pigmented layer removed from the mid-dorsal region of 5 animals. The fragments cut off slowly curled up at the edges, concealing the cut surface; this occupied about thirty seconds. The covered area was again exposed by carefully unrolling the curled-in parts, after which they slowly curled in again (1,5 min.), but not so completely as before. The wound on the body was temporally closed by a sharp bend on the long axis at the injured spot, such that, with the assistance of a deep contraction of the transverse muscles at that point, the scar was completely hidden.

9,50 P.M. Body was now straightened out, and the wounded surface noticeably smaller in area.

July 31. 6,00 A.M. Edges of the wounds now indistinct, and the scar surface had become covered over by a thin yellow epithelium.

Pigmentation slowly increased, but even after three weeks the injured areas could still be seen as light-colored spots.

Subsequent experiments dealt with the phenomena of inrolling of the cut edges and regeneration, and are recorded as follows:

Exp. 73,1. Slits cut in the body-wall.

July 14. Three animals prepared with a lateral incision, perpendicular to the long axis, made with sharp scissors. Parts of the respiratory trees, extruded through the cuts, later withdrawn (cf. Torelle, 1909). Body turgor lost (but recovered by the next morning). No disturbance of locomotion. A deep ring constriction at the level of the cuts, together with a sharp bend at that place, closed the wound. The edges of the wound tucked in within 5 minutes.

July 17. Cuts healed over, only a slight scar showing.

Exp. 29,2. Animals slit open longitudinally from mouth to anus.

Isolated pieces show a strong tendency to curl in at the cut edges; this is well illustrated when the body is cut from anus to mouth along the dorsal interradius, as the edges of the cut then roll in tightly, so that the bodywall is coiled in a double scroll.

Exp. 41,1. Anterior end removed.

July 7, 9,00 A.M. Anterior end, including the calcareous and nerve rings etc., removed from 5 specimens; length of pieces removed, 4,8—6,5 mm.

8,30 P.M. Cut ends all closed over, and the "decapitated" pieces crawled about in normal fashion, though slowly.

July 8. 9,00 A.M. The two smaller amputated pieces dead.

July 9. 9,00 A.M. One other anterior piece dead.

July 12. 12,00 M. The two remaining anterior pieces showed a bulging posterior regeneration cone. Preserved in alcohol.

July 14. 9,00 A.M. A similar regeneration cone noticed on the anterior ends. All regenerated parts very pale in color.

July 19. 8,30 A.M. Regeneration cones 4—6 mm. long on the new anterior ends; most of the time they were kept well invaginated. The cones were of much less diameter than the rest of the body. In two cases 11 tiny new tentacles were counted, together with several papilliform growths, evidently representing the fundaments of other tentacles; in another specimen 9 well formed tentacles, 1 very small one, and 8 stumps were counted. The new tentacles were thin and pale, and approximately 2 mm. long.

July 22. Extreme length of regenerated pieces, 1,0—1,3 cms.

Exp. 72,1. Posterior end removed.

July 14. Posterior ends, 5 mm. long, removed from 5 animals. The new posterior ends rolled in almost immediately (5 min.); the amputated pieces closed the wounded end in 10 min.

July 15. A distinct opening at the new posterior ends.

July 17. Small regeneration cones visible.

July 20. The new integument thin and bulging, regeneration cones 3—4 mm. long.

July 22. All dead, through accidental stagnation of the water.

Experiments in which cuts were made with purposely ragged outline, and in which pieces were removed by slanting cuts, gave results not essentially different from those already described. Wound closure was more successful when the cuts had smooth edges, as otherwise the ragged parts were sloughed off; slanting cuts were closed in exactly the same manner as those made perpendicular to the long axis of the body, but the thinner part of the cut end was also sloughed off in most instances.

Some tests were made with animals kept in M/800 chloretone, in the hope of eliminating by this means the action of the muscles in bringing about the inrolling of the wound edges. It was found that with chloretoned animals the rapid in-bending did not occur, though after an hour or more the edges of transverse cuts were slightly curved. It would therefore seem that the direct action of the injured muscles plays a more prominent part in wound closure in *Holothuria* than it does in actinians (RAND, 1909; CHESTER, 1912).

Holothuria surinamensis can therefore regenerate, or rather reform from its body-tissue (for the operated animals were kept in aquaria without sand or mud to provide nutritive material) an anterior or a posterior end within a very brief interval after the removal of those parts. The significance of this power, as favorable to a method of reproduction by self division, has already been pointed out (page 243). The incomplete restoration of the usual complement of tentacles would account, in part at least, for the numerical variation of these structures found in some specimens.

III. **Mechanical stimulation.**

Echinoderms are known to be generally sensitive to a variety of mechanical stimuli. The experiments of Romanes (1885), Preyer (1886), Jennings, (1907), Cowles (1910), et al., have shown that tactile and pressure stimuli play an important part in the behavior of starfishes and ophiuroids. The apodous holothurians possess a highly developed tactile sense (Clark, 1907, p. 47, 138). In addition to touch papillae, synaptids have positional organs (Clark, 1901a, 1907, Becher 1909), operating through mechanical stimulation after the fashion of the molluscan and crustacean statocyst, which are apparently irresponsive to vibrational stimuli. The tentacles and podia[1]) of pedate holothurians are usually spoken of as "tactile organs", and the general surface of these animals is also sensitive to pressure stimuli (Henri 1903a, b, c; Grave 1905; Pearse 1908).

1. Local mechanical stimuli

were applied to *H. surinamensis* by the use of needles, finely drawn out glass rods, and minute bubbles of air formed at the tip of a capillary pipette.

a) The tentacles were very sensitive to slight touches. A very slight stimulus led, in a resting animal, to the collapse of the tentacle stimulated and its withdrawal; a more severe one, or a weak stimulus repeated three or four times, produced complete retraction of all the tentacles with or without an accompanying turning of the anterior end to one side — the side away from that stimulated. The reaction time was 0,3 sec.[2]), at 24—27°, and was (as nearly as could be determined) constant for all intensities of stimulation, though the amplitude of response varied in proportion to the severity of the stimulus. The tentacles also responded in this way to fairly strong water currents from a pipette. When all the tentacles had contracted as the result of a stimulus applied to a single one, the tentacle stimulated was the last one to reappear when the set was reëxtended.

The tentacle reflex to mechanical stimuli affords a very clear instance of the relation between a physiological state and reaction, for when holothurians actively engaged in locomotion were studied, it was found that the tentacles were apparently much less sensitive than when in the resting condition; in fact, even relatively strong stimuli produced merely a waving about and partial retraction of the particular tentacle affected, while the others were markedly extended.

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[1]) As a matter of convenience, I shall use the term "podia" to include merely the pedicels and papillae.

[2]) All the measurements of reaction time recorded in this paper were made with a calibrated stopwatch reading to 0,1 second.

Considerable variation was found in the amplitude of response obtained from the tentacles of different individuals; with some even the slightest stimulation leading to a maximum effect which is secured in others only by repeated stimulation. After stimulation with solutions of various chemicals the threshold for the mechanical stimulus was considerably lowered, a maximum amplitude of response then being obtained with the slightest touch.

No differences could be detected in the reactions of the different tentacles, nor in different regions of the same tentacle, though it might have been expected that the outer surface of the discs would have been more sensitive than the shafts.

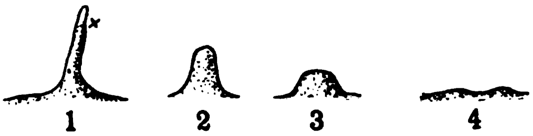

Fig. A. Stages in the contraction of a papilla (2:1).

1. A fully extended papilla. 2. First stage in contraction — response to a slight touch, at *x*. 3. Second stage in contraction — response to more intense stimuli. 4. Complete retraction of papilla repeatedly stimulated.

b) The podia. As nearly as could be determined, the pedicels and papillae were of equal sensitivity to tactile stimuli. In both cases the reaction time of podia in the mid-body region was slightly greater than that of the tentacles, being 0,4—0,6 seconds at 24°—27°; for stimuli of approximately equal intensity the amplitude of the response was much less than that of the tentacles in non-moving animals.

The papillae are more scattered than the pedicels and could readily be studied singly under a binocular microscope. They showed several stages of reaction to tactile (and other) stimuli. In general, the mode of retraction was by contraction; but the extent of the retraction varied with the intensity of the stimulus, and the following well-defined steps were made out (Fig. A): (1) shows the outline of a fully extended papilla; when gently touched, at x, the thinner distal portion, yellow in color, collapsed through the withdrawal of water into its ampulla and the contraction of its longitudinal walls, and the outline seen in (2) was assumed; more vigorously stimulated, the contraction proceeded to such an extent that only a slight elevation remained upon the skin, (3); the final step, involving the complete invagination of the papilla, could be secured only on repeated stimulation; in this last stage (4) a small hole in the integument was all that was seen from the outside.

Mechanical stimuli of a more vigorous kind, such as gently pinching with forceps, called forth reactions varying with the region of the body concerned. On the most anterior part of the animal, within a centimeter or so of the tentacle ring, pinching a papilla led first of all to a contraction of the tentacles and collar, and of the longitudinal muscles in such a fashion as to bend the anterior end of the body away from the side stimulated, and then both the affected papilla and its immediate neighbors would retract. If the stimulated papilla was dorsal in position, the anterior end was bent ventral and more or less beneath the body. To fairly severe stimuli nearby papillae also reacted by slight contractions. The tentacular brim was extremely sensitive to touch, its reactiveness being comparable to that of the tentacles. In the mid-body region, pinching a papilla led to the contraction of nearby papillae also, especially those on approximately the same transverse circumference. Further evidence of the conduction of stimuli in circular fashion about the mid-body was given by the transverse-muscle contraction, which appeared as a narrow ring-like depression at the level of a vigorously stimulated papilla; this type of response was usually not accompanied by any sidewise turning of the anterior end. In the region within a centimeter of the cloacal tip, pinching a papilla produced a reaction of the posterior end comparable to that already described for the anterior end, but less rapid and of not so great an amplitude. The papillae forming a ring about the cloacal brim were much more sensitive to tactile stimuli than the dorsal and lateral podia; stimulation here effected a closure of the cloacal aperture and more or less of a sidewise turning of the posterior end of the body.

The tube feet reacted to pinching by contraction, and neighboring pedicels in an antero-posterior line running through the stimulated pedicel also reacted; usually, those anterior to the point of application of the stimulus reacted more quickly than the posterior ones, and the disturbance was generally carried farther in the anterior than in the posterior direction. By severe treatment, the tube feet were caused to become retracted completely, as in the third contraction stage described for the papillae (Fig. A, 4). Attached tube feet required much greater stimulation to induce their contraction than did free ones. Tube feet were allowed to become attached to glass rods, and were then gently pulled; this in most experiments led to contractions at the anterior and posterior ends of the animal, and ultimately to the freeing of the tube foot, even with very slight tensions. The tips of the papillae and the terminal discs of the pedicels were much more sensitive than their stalks.

Stimuli applied to the lateral walls of papillae and pedicels gave results which are of interest in view of von Uexküll's theory of the action of the nerve net system in echinoderms. When the side of a papilla not exactly perpendicular to the body surface, i. e. in which the muscles were more contracted on one side than on the other, was touched with a finely drawn out glass rod, or gently pressed upon by a small bubble of air at the tip of a pipette, the resultant reaction involved two steps — (1) an erection of the papilla, and (2) its collapse and contraction. This led to the reaction appearing as a positive one when the less contracted side was touched, or a negative one when the more contracted side was stimulated. The two steps were often fused together, but I believe that we have here something parallel to the state of affairs at the base of an Echinoid spine, where v. Uexküll (1900a) found the stretched muscles to be more easily and quickly thrown into contraction.

c) The body surface at the anterior and posterior extremities was much more sensitive than that in the mid-portion of the animal's length. In the region within a centimeter or so of the anterior end (varying with the size of the individual), a slight touch on the dorsal or lateral surface between the papillae produced a reaction from the tentacles, brim, and nearby papillae, and some longitudinal contraction of the body, together with more or less turning to the side away from the point of application of the stimulus. The mean of ten determinations gave an average reaction time of 0.4 sec. (at 26°) for delicate tactile stimuli. The drosal and lateral surfaces in the middle region were relatively insensitive. To slight touches no response whatever was secured, unless the particular area had been made hypersensitive by chemical stimulation immediately before the test, for example, as, by three or four applications of M/50 acid.1) The body wall might be forced in over an area of about a square centimeter by a water current from a pipette without producing any reaction. On severe prodding a rather complicated series of reflexes was produced: a ring-like depression, deepest at the point stimulated and usually disappearing on the ventral surface, was accompanied by a reaction from the anterior end such as I have previously described under the responses to pinching a papilla. Individuals resting on the bottom of an aquarium with a curve or bend in the long axis were studied, and it was found that whether the stimulus was applied on the inside or outside of the curve,— i. e. to either the more contracted or the stretched side,— the bending away of the anterior end was such as to carry this part away from the side stimulated.2) An exception to this general statement was found in a somewhat different form of reaction, in which the pedicels, when stimulated in the manner above described, released their hold and the animal rolled over on to the side opposite that stimulated; if stimulated again, as they lay on one side, the bending of the anterior and posterior ends was such as to bring them towards the spot touched. The latter type of reflex depends upon the fact that the ventral musculature is much more effective in producing body curvature than the dorsal. The ventral and dorsal surfaces were of very nearly the same sensitivity. The region in the immediate neighborhood of the cloaca behaved in a manner similar to that about the mouth, a slight touch leading to the closure of the cloaca, longitudinal contraction, and some turning to the unstimulated side. Reaction time (at 25°) averaged 0,6 seconds.

When picked up and handled, or otherwise subjected to repeated mechanical stimulation, *Holothuria* decreased markedly in volume. Strong contraction of the longitudinal musculature forced out through the cloaca water previously held in the respiratory trees, producing a decrease in length to little more than half its value in expansion, with only a slight increase in the maximum diameter (Table II). In this process the pedate surface remained flattened, while the dorsal

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1) Jennings (1907, p. 69) found a similar interrelation between mechanical and chemical stimuli in the starfish.

2) This bending away of the anterior end involved, of course, the release of the pedicels in that region.

surface became strongly arched and the two ends much narrowed. The relatively greater strength of the trivium muscles was shown also in the way in which holothurians detatched from the substratum and lying on one side became curved dorsoventrally, the ventral surface occupying the inner curvature. Exactly opposite conditions hold with *Caudina*, *Thyone*, and other forms (GEROULD, 1896; PEARSE, 1908, et al.), where the dorsal muscles become more powerfully contracted when the animal is not attached to a horizontal base.

Table II.

Showing relative sizes of holothurians expanded and in contraction.

No.	Normal		Contracted	
	length cms	diameter cms	length cms	diameter cms
1	15,5	2,0	10,0	2,5
2	10,0	1,4	4,0	2,1
3	16,5	2,1	8,9	3,2
4	19,1	1,9	9,0	2,2
5	18,0	1,8	8,6	2,5

d) Experiments on mutilated animals. Small isolated anterior and posterior ends, and the two parts of bisected animals, reacted normally to tactile stimuli, except in the immediate neighborhood of the cut surface. The responses of tube feet and tentacles on isolated parts were of very slight amplitude, as compared with those of the normal animal, but otherwise no differences could be detected.

e) Discussion. The reactions of *H. surinamensis* to tactile stimuli may be summarized as follows:

All parts of the body are sensitive to touch, the order of responsiveness being tentacles, oral brim>cloacal brim>podia> anterior end>posterior end>mid-body surface. As in many other animals, the anterior and posterior ends as a whole are the most sensitive regions, and the anterior one is the more reactive. The tentacles and podia (including the cloacal papillae) are the chief organs of the tactile sense.

The end-organs concerned in the reception of tactile stimuli cannot be specifically designated. In synaptids (CLARK, 1907, p. 47) the so-called touch papillae are small groups of epithelial cells connected with ganglia at the ends of branches from the tentacle or radial nerve strands; sensory cells of presumably similar function also occur scattered over the body of synaptids, and in the general epithelium of *Molpadiidae* (CLARK, 1907, p. 154). CLARK suggests that the cloacal papillae of the *Molpadiidae*, like the tentacle digits in this and other Apodous groups, are important organs of touch. *Cucumaria* and *Holothuria* have no special form of nerve termination even in the tentacles (CUÉNOT 1891, p. 515; RETZIUS 1906), but the podia and tentacles are each provided with an extension of the nearest radial or tentacular nerve strand ending in a plate of sensory epethelium at their distal terminations. POLARA (1906b), however, has found what may be (?) tactile organs, in the integument of *H. tubulosa* and *H. poli.*

HENRI (1903a, b, c) describes reactions of *Stichopus regalis* which point to the existence of ramifications of the radial "nerves," each point on a longitudinal muscle being reflexly connected with a series of points on the body which he finds located within a certain limited region. He accounts for the different reactions obtained from the anterior end near the mouth, by saying that here the nerve-ring apparatus enters, and through its action as a conductor a pair or more of the longitudinal muscles contract. He concludes that the nerve ring acts as a central nervous organ. I have incidentally verified some of HENRI's observations on the mechanical stimulation of the body-wall of *Stichopus* (*S. moebii*) cut open along an inter-radius. The mode of reaction of the mid-body wall of *Holothuria* to tactile, photic (p. 261) and chemical (p. 281) stimuli, leads me to believe that the nervous system of *Holothuria* works in a manner agreeing with HENRI's description. But it must be noted, on the other hand, that animals from which the "central nervous organ" had been removed were still capable of coördinated activity, — of moving away from the light, climbing vertical walls, and turning the anterior end away from the side stimulated,— though they appeared to be in a much weakened condition. COLE (1913b) believes that a starfish with its circumoral nerve ring cut cannot effect coördinated righting movements, but experiments shortly to be described show that this is entirely possible for *Holothuria,* and I therefore do not believe that the nerve ring necessarily acts as a true central nervous organ in HENRI's sense.

Furthermore, the posterior end of *Holothuria* exhibits these sideward bendings, etc., and no trace of an aboral nervous ring has been identified among the holothurians.

2. Continued mechanical stimuli as a factor in the behavior of *Holothuria* was sought in evidence for stereotropic and similar forms of irritability.

a) Stereotropism; and the righting reaction. Holothurians were sometimes found to have lodged themselves in narrow holes and crannies in rocks, and this, taken in connection with the burrowing habit, seemed to point to a general stereotropic sensitivity of the animal's surface. In aquaria however, they showed no tendency to collect in groups, not even in the angles between walls or in the corners. When fitted into narrow glass tubes so that the anterior end projected through one opening, they did not tend to maintain this position, but soon crawled out of the tubes. Similarly, experiments designed to discover the existence of rheotropism in *Holothuria* gave negative results. The animals moved either with or against the current flowing down a narrow trough, depending on circumstances not connected with the current itself, save when the water current was violent, as then the effect was comparable to strong mechanical stimulation, producing sideward turning away of the anterior end and gradual movement until outside the zone of disturbance, but without any orientation.

Echinoderms generally are capable of righting themselves when turned over on the dorsal surface. It is known that the righting reaction of the starfish is due to the stereotropism of its tube feet, rather than to any geotropic control of the dorso-ventral orientation of the body (MOORE, 1910). Synaptids possess definite "positional organs", which function as statocysts, but no such organs occur among other holothurians, and the following experiments show that *Holothuria* in its righting behavior is comparable to the starfish — or better, to an isolated starfish arm.

The righting reaction of *H. surinamensis* involves two distinct factors — 1. the dorso-ventral curvature of the body, and 2. the twisting of the animal on its long axis, forward crawling, and the gradual attachment of its pedicels to the substratum. The previously described tendency to throw the body into a curve when turned over on its back or otherwise removed from its normal position makes it impossible for *Holothuria* to remain on its dorsal surface, but causes it when so placed to topple over to one side or the other. Then, beginning always at the anterior end, the body becomes slowly twisted in such a way as to bring the pedate surface to the bottom; the body muscles are more important in this procedure than the tube feet. This type of righting behavior is very like that practised by planarians, slugs, earthworms, etc. A modification of this method of righting was used in a few instances: the anterior and posterior ends would for a moment bend ventrad rather quickly, and the animal thus was enabled to roll over suddenly with sufficient momentum to regain its normal orientation without the use of longitudinal twisting.

Holothurians with the anterior end (nerve ring, stone ring, etc.) amputated did not right themselves until several hours after the operation. This was probably due to the inability to develop internal water pressure in the usual way; but sooner or later the "headless" pieces became righted after the manner above described for the normal animals. With animals cut cross wise into two equal parts, the righting movements of the posterior half began at the tail end — showing a condition somewhat comparable to the non-use of injured arms (starfish — MOORE, 1910) or injured regions (sea urchin — ROMANES, 1885) in other echinoderms. COLE (1913b) concluded that a starfish could not coördinate its righting movements in the absence of a complete nerve-ring; it is probable that the simpler structure of *Holothuria* makes possible a better coördination through the mutual pull of the body parts than is the case in *Asterias*. Some tests were made with holothurians suspended in the water by a thread through the dorsal integument. Two types of behavior were seen in such animals; some individuals squirmed about incessantly, while others assumed a contracted form and kept it as long as the experiment was continued (3—4 hours), in one case autotomizing the viscera. Detached tube-feet, and the tube-feet of holothurians held resting on their dorsal surface, readily became attached to the surface of bits of stone etc., but not so frequently to glass rods. Direct contact with a rough surface is the stimulus which provokes their attachment. *H. surinamensis* was sometimes collected from the under side of slabs of rock, and this fact, taken in conjunction with those already described, argues for the conclusion that the normal righting reaction depends on the positive stereotropism of the tube feet. When the pedicels are detached the animal assumes such a form as best to make possible their re-attachment. Similar righting behavior was observed in other species of *Holothuria* and in *Stichopus*.

b) The climbing of vertical walls. All the holothurians which I have studied were frequently found near the water surface, on the vertical walls of aquaria in which they were confined. A holothurian of different type, *Cucumaria cucumis* of the Bay of Naples, supplied LOEB with a well known instance of negative geotropism (LOEB, 1891), but *Thyone* (PEARSE, 1908, p. 275) does not show any noticeable geotropic tendency. From the fact that (save possibly in the case of *Stichopus*) crawling up a vertical surface did not involve any orientation, I was led to believe that, with *H. surinamensis* at least, geotropic irritability was not necessarily the responsible factor. In crawling up the wall of an aquarium the long axis of the animal was not placed vertically, but always at an angle with the horizon, and the completion of the movement at the surface of the water found the body placed horizontally immediately under the surface film. Frequently the circlet of tentacles was expanded and moved about under the surface film; during this maneuver a few of the most posterior pedicels were sufficient to hold an animal in its position. Experiments were made with holothurians placed upon glass plates tilted at an angle of 30° to the horizon; in these tests approximately equal numbers crawled upward and downward, while many remained stationary, the actual figures being: 6 upward, 5 downward, 9 stationary in 20 tests. Not all the specimens in an aquarium were at any one time to be found on its sides; some came to rest in shaded corners and remained on the bottom, while others crawled down from the water surface after several hours.

JENNINGS (1907, p. 115), COWLES (1910, 1911a), and COLE (1913a) have called attention to the persistence of the impulse to locomotion in starfish and ophiuroids, and COWLES ascribes the climbing of vertical walls by *Echinaster* to this peculiarity of behavior. The activity of *Holothuria* was very similar in this respect to that of *Echinaster* as described by COWLES (1911a), for, when oriented by light or when engaged in continued locomotion from other causes, this form also continued its crawling by mounting such vertical or inclined surfaces as it met in its path. This was very noticeable among animals freshly collected, for here the disturbance due to handling and stimulation by light invariably resulted in the crowding at the surface of all the holothurians in a pail. The absence of geotropic irritability is also indicated by the fact, already referred to, that *Holothuria* was found ventral side uppermost clinging to the under faces of rocks.

The presence of the nerve-ring is not essential for the climbing of vertical walls. While regenerating new anterior ends the holothurians were for the most part quiescent, but they occasionally moved slowly about and were sometimes found to have crawled to the water surface, assuming the position of normal individuals.

3. Vibratory mechanical stimuli.

Holothuria responded to slight jars of the table on which the aquaria were placed by momentarily withdrawing the tentacles, and ceasing the rhythmic cloacal movements. Tests were made by placing holothurians in beakers of thin glass, three quarters filled with sea-water, and tapping gently on the lip of the beaker. After a certain number of repetitions of this stimulus, no response was given. Table III gives, for ten experiments, the number of jars required to bring about this state of accomodation; the tapping was done with a glass rod, the successive taps being one minute apart and of as nearly equal force as possible. The extent of the reaction obtained from different individuals was quite variable. Animals designated b and h in the Table ceased to react, after giving the number of responses noted, by withdrawing the tentacles permanently, the others by keeping them extended. While no withdrawing reflexes on the part of the tentacles were obtained after tapping for some little time, continued jars induced restlessness and locomotion.

Table III.

Exp. 46. 1,2	Reactions to jarring									
Individual	a	b	c	d	e	f	g	h	i	j
No. of reactions	4	2	6	9	6	3	12	3	4	8

Note. The "No. of reactions" given is the number required to bring about accomodation in each case.

Similar results were obtained by allowing drops of water to fall from a height of 20 centimeters, approximately three centimeters of water being over the animals. The response here was ocalized, the tentacles, mid-body region and posterior end all giving the usual withdrawing reactions when the drops were made to fall over them.

Holothuria with the anterior parts amputated gave the same series of reactions to vibrations, but were more easily exhausted, not more than 5 successive reactions being obtained to shocks coming at one minute intervals.

IV. Light.

1. Introduction.

The radial arrangement of the body in starfishes and sea urchins has given a special interest to the study of their reactions to light in connection with theories of phototaxis which have been advanced on the basis of work with bilaterally symmetrical animals. In echinoderms other than holothurians there is no good evidence of orientation produced by light. With regard to the holothurians MAST (1911, p. 211) says: — "The lack of orientation in moving from a source of light is much more striking in the holothurians, which are superficially at least much more definitely bilaterally symmetrical" than the starfishes; this generalization is based on PEARSE's (1908) account of the behavior of *Thyone*. So far as known the holothurians are uniformly negative in their reactions to light, and since the time of DALYELL (1851, Vol. I) have been spoken of as nocturnal animals; *Stichopus* is an apparent exception, for it is seen in great numbers on brightly illuminated bottoms, but even here some species at least crawl into dark places after the passage of the breeding season (MITSUKURI, 1903).

2. Phototropism.

Holothuria surinamensis, captiva and *rathbuni* all move away from a source of light, in a manner strikingly at variance with that described for *Thyone*. As has been brought out in the discussion of locomotion (page 242), *Holothuria* was not observed to crawl in any other way than with the anterior end to the front, while *Thyone* moves away from the light, like a sea urchin (HOLMES, 1912) with any angle of the body in advance. When a number of holothurians were placed in a vessel near a window, though not exposed to the direct rays of the sun, they oriented away from the light and moved to the side of the vessel farthest from the window. Having gotten this latter result was obtained when the beam of light was so narrow that the forward extension of the anterior end carried the tentacles out of its path. The usual result of these tests was that, after some preliminary extension of the anterior end, it was swung sharply away from the lighted side; following this, contraction of the longitudinal muscles in some cases brought the whole animal out of the path of the light, while in others locomotion in the direction of the anterior end led to crawling along the path of the light beam. In this latter form of response, any tendency to enter the shadow was checked by the prompt retraction of the tentacles and the customary "avoidance reaction" when the tentacles were shaded, even by the animal's own body. Tests in which the light was limited to the brim region, the tentacles being in the shade, gave identical results.

b) When unilateral light was allowed to fall on the mid-body region, holothurians moved straight ahead into the dark. There was no perceptible orientation until the light struck the most posterior centimeter and a half of the body, which was then turned away from the light, but the direction of locomotion was not affected. When so illuminated that several centimeters of the tentacle end were shaded, the same result was obtained — forward motion without orientation.

c) Unilateral illumination of the posterior tip caused it to be thrown sharply away from the light, but in a manner indicating, both by the reaction time and the amplitude of the reaction, that the posterior end was less sensitive than the anterior one.

In all these experiments the illuminated region appeared to be locally constricted — a result to be expected from the previous tests with a spot light.

Exp. 61,2—62,1.

Horizontal light parallel to the long axis of the body was thrown on the anterior end. Result: a contraction of the longitudinal muscles, greater on one side than on the other, such that subsequent forward locomotion carried the animals outside the field of stimulation.

Exp. 62,4.

Five holothurians deprived of the tentacles and ring structures did not move about to any extent until a regeneration cone had made its appearance. Such animals were just as strongly negatively phototropic as normal ones.

Placed in a field of light coming from one side only, the reaction uniformly obtained from *Holothuria* was a turning of the anterior end away from the side illuminated, and continued locomotion away from the source of light in the direction of its rays.

Holothuria surinamensis is therefore without question negatively phototropic.

3. The shadow reflex.

In the course of several hundred trials, no reflexes other than the slow system of movements producing negative phototropism were observed when the intensity of the light falling on *Holothuria* was suddenly increased. This was true for individuals which had been in darkness for some hours, as well as for those which had been in light of low intensity for even longer periods. The partial retraction of the tentacles when illuminated by a spot light was much slower than the shadow reflex presently to be discussed, and was not of a definitely predictable character; it appeared for the most part as a general waving about of the tentacle stimulated.

All the holothurians which I have examined give, however, a well defined response to sudden diminution of light intensity, a reflex which forms a feature of the behavior of other holothurians also, e. g. *Cucumaria* (Grave, 1905) and *Thyone* (Pearse, 1908). Shading the whole animal produced retraction of the tentacles, closure of brim and cloacal sphincter, and a general contraction, especially of the anterior and posterior ends. Following this, the parts thus contracted would soon expand, and remain expanded in the shade. The shadow reaction was most easily studied in the case of the tentacles, where the reflex consisted of the withdrawal of the tentacles, the closure of the brim, and a bending of the body to the side away from that shaded. The extent to which the tentacles retracted, the time during which they remained concealed if complete withdrawal had been secured, and the degree to which the body muscles were implicated in the reaction, were in a measure determined by the area of the region shaded, the sharpness of the shadow edge (i. e., the rate of intensity reduction), and the actual amount of light reduction accomplished by the shading. Single tentacles were caused to disappear within the partially constricted collar by shading them individually, either at the peltate tip or at any point along the shaft. I did not determine the minimum shadow area which would produce this reaction.

The whole surface of *Holothuria* is sensitive to sudden shading. With the tentacles completely retracted and the brim closed down, well defined reactions were obtained from the anterior end, especially from the brim. The rim of the cloaca was particularly sensitive; very slight reactions were had from the posterior end when this rim was concealed by the closure of the cloaca, or when it was amputated. The relative "shadow sensitivity" of the various parts studied is roughly shown by the reaction times in Table IV. The relative amplitudes of the reactions are indicated in decreasing order by the numbers (1) . . . (5). The reaction times are the averages of ten sets of observations. The variability of these reactions, as obtained from the numerous animals observed, was very great.

Table IV.

Reaction times to sudden shading — in seconds. Temp. 27,5°.

Tentacles	Ant. end 1)	Post. end	Mid-body 2)	Podia 3)
(1)	(2)	(3)	(4)	(5)
1,2−	2,0	1,0−	2,0+	∞ ?

1) With the tentacles completely retracted.
2) Only in bright sunlight, and not even then in all animals.
3) Response too indefinite to be measured.

Of all the reactions those of the tentacles were the most clearly defined, and in the experiments made to determine some of the characteristics of this reflex the tentacles were studied exclusively.

It may first be pointed out that the tentacles on small anterior fragments which had successfully accomplished the closure of the cut end, reacted to shadows in an entirely normal fashion, both as regards the reaction time, and the period during which the brim was closed down, though these isolated pieces were very quickly exhausted (see Tables V and VII).

Table V.

Exp. 27.3.

July 2. Comparison of the shading response of normal tentacles, and of those on isolated anterior pieces. Anterior ends removed 40 hrs. before this experiment. Measurements in seconds. Temp. 25,0°.

No.	Normal		Isolated ant. ends	
	Reaction time	Period of contraction	Reaction time	Period of contraction
X	1,3	10,0	1,9	16,0
Y	2,8	2,0	3,0	18,0
Z	2,0	5,0	2,4	2,5

Normal holothurians in well aerated sea water were stimulated by successive shadows cast by a small opaque black board, in direct sun light, in less intense light away from the direct rays of the sun, and, during some experiments, in lamp light. Table VI contains successive reaction-time measurements of the tentacle reflex to

Table VI.

Exp. 4.38.1.

July 2. Successive reaction times of ten normal holothurians in the shadow reflex. Temp. 23,0°. Diffuse light. Shadows 0,5 min. apart.

No. of stimulus	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	1,3	1,0	1,2	0,6	0,4	2,1	2,8	1,4	2,5	0,5
2	1,7	1,1	1,8	1,6	1,2	2,4	2,4	1,2	2,3	1,6
3	2,8	1,5	2,9	1,4	1,6	1,0	1,0	1,6	2,4	2,4
4	2,2	2,0	1,0	1,4	1,0	1,5	2,0	2,4	1,5	0,8
5	1,8	3,0	1,6	1,4 1)	0,8	2,4	3,0	3,2	0,9	3,2
6	2,0	2,5	1,4	∞	1,5	2,6	3,2 1)	0,6	2,0	∞
7	1,8	2,7	1,0	∞	3,2	1,1	∞	1,6	4,5	∞
8	1,4	1,6	1,5	∞	2,1	1,6	∞	1,6	∞	∞
9	1,6 2)	0,8	2,4	∞	∞	3,5	∞	1,4	1,3	∞
10	2,0	1,4	2,6	∞ 3)	2,4	1,8	∞	2,5	2,4	∞
11	1,0	1,8	0,6	.	1,3	2,4	∞ 3)	2,8	1,2	.
12	∞	2,4	1,0	.	1,0	2,3	.	5,4	0,8	.
13	1,1	1,0	4,5	.	1,5	6,1	.	∞	1,6	.
14	1,6	1,4	∞	.	4,2	2,7	.	∞	2,4	.
15	2,6	1,6	∞	.	3,9	1,3	.	∞	3,9	.
16	1,8	2,5	2,1		4,5 3)	1,0	.	∞	6,0	
17	2,3	3,1	∞		∞	3,1		∞ 3)	∞	
18	∞	2,7	∞		∞	∞		.	∞	
19	2,3	1,4	∞		∞	4,6		.	∞	
20	6,4	2,2	.		∞	∞		.	∞	
21	2,8	1,3	.		.	5,1		.	∞	
22	3,6	∞	.		.	∞		.	.	
23	1,8	∞	.		.	∞			.	
24	1,8	∞	.		.	∞			.	
25	∞	∞ 3)			.	.			.	
26	∞	.				.			.	
27	∞	.				.				
28	∞	.				.				
29	∞	.				.				
.	.	.								
.	.									
.	.									
.	.									
.	.									
37	∞ 3)									

Notes.
∞ Indicates "no reaction" (i. e. infinite reaction time).
1) Tentacles remained withdrawn.
2) From this reaction on, the amplitude of the contractions decreased.
3) One half hour later, had recovered and gave good reactions.

shadows cast every 0,5 minute, in the diffuse light of the laboratory. A similar table for the isolated tentacular ends is shown in Table VII. The shadows in each case lasted approximately one second, though their actual duration was of no consequence for the experiment. It will be seen that the rate of exhaustion varied greatly among the different individuals. Two of the animals (Nos. 4 and 7) ceased to react with the tentacles retracted, while all the rest remained expanded. A feature of this reaction, which the plot in Fig. B brings out more plainly, is the rhythmic character of the exhaustion curves. The sensitivity to shadows first falls off to some extent, then increases in recurring periods of some regularity, finally disappearing altogether, but returning after a short interval of rest.

Table VII.

Exp. 27.3. Shadow reactions of five (a—e) isolated tentacle-bearing parts.

June 30. 4,00 P.M. 1,5 cms of anterior end removed from each of 5 animals ranging in size from 5,0 to 8,5 cms in length.

July 2. 9,00 A.M. Tentacles expanded; the small anterior pieces had successfully closed over the cut, and three of them were moving about with the aid of their tube feet.

Shaded at intervals of 1 min., in diffuse light, the shadows lasting 1± second. Temp. 26,0°. Reaction times in seconds.

No.	a	b	c	d	e
1	1,0	1,4	2,1	1,2	1,5
2	3,4	1,7	1,3	1,0	1,8
3	2,1	3,0	3,1	1,1	2,1
4	1,7	2,4	1,0	2,4	1,8
5	∞	1,8	∞	3,4	2,5
6	∞	1,6	1,8	1,8	3,2
7	∞	1,6	∞	1,3	1,0
8	.	∞	1,4	1,0	1,0
9	.	1,6	∞	∞	∞
10	.	1,0	∞	∞	∞
11	.	∞	∞	∞	∞
12	.	∞	∞	∞	∞
13	.	∞	.	.	.
14	. .	∞	.	.	.

Note. ∞ Indicates "no reaction" (i. e., infinite reaction time).

When the tentacles retracted as the result of shading, they remained concealed for a length of time which may be taken, in a certain sense, as a measure of the amplitude of the shadow reflex. Table VIII gives the successive "retraction times" obtained from three holothurians in bright sunlight. The stimuli were applied every half minute.

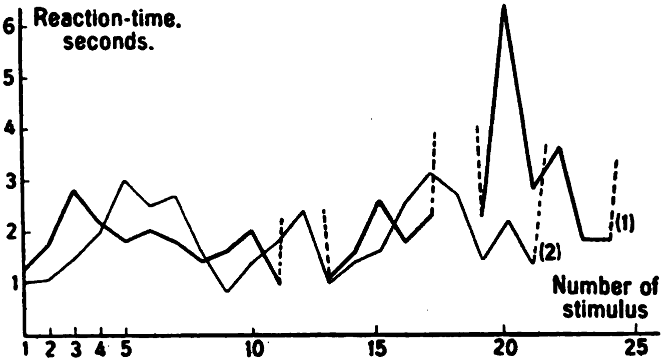

Fig. B.
Reactions to repeated shading — 0,5 min. intervals (Individuals (1) and (2) of Table VI).

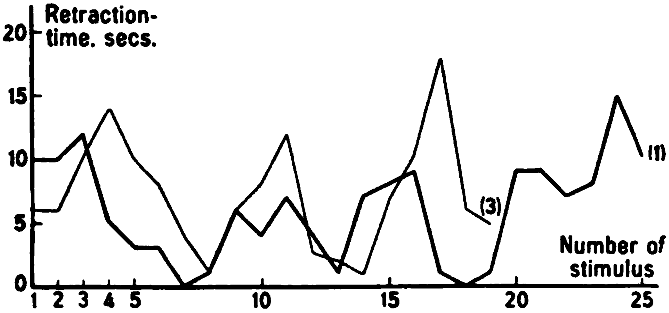

Fig. C.
Amplitude of the reaction to successive shadows, 0,5 min. intervals; "retraction-time", seconds (individuals 1 and 3 of Table VIII).

The observations of Table VIII are shown graphically in Fig. C. Comparing this with Fig. B, for the reaction times to successive shading, it will be seen that the curves of both the reaction time and of the amplitude of the reaction as measured by the period of tentacle concealment, show the rhythmic character to which attention has already been called.

Table VIII.

"Retraction times" (seconds) to shadow stimuli.

Exp. 42. July 7.

Temp. 29,0°. Bright sunlight. Shaded every half minute. Time given only to the nearest whole second, because of the difficulty of obtaining accurate points for measurement.

No. of stimulus	(1)	(2)	(3)
1	10	8	6
2	10	8	6
3	12	7	10
4	5	12	14
5	3	13	10
6	3	9	8
7	0	4	4
8	1	0	1
9	6	0	6
10	4	5	8
11	7	0	12
12	4	3	3
13	1	1	2
14	7	0	1
15	8	0	7
16	9	0	10
17	1		18
18	0		6
19	1		5
20	9		0
21	9		0
22	7		0
23	8		
24	15		
25	10		

The shadow reflex was given only within a certain range of temperatures. Holothurians were heated in seawater contained in glass beakers and the temperature at which the shadow reaction ceased was noted. The record of one such experiment is here given:

Exp. 38. July 5.

Heated in bright sunlight; 10 animals used.

Time	Temp.	Observations
12 : 21,5	30°	Shadow reaction normal.
12 : 25,5	31°	Tentacles not completely withdrawn when shaded.
12 : 30,5	32°	Tentacles merely waved about gently when shaded.
12 : 37	32°	Same.
12 : 43	34°	No shadow reaction at 34° or above.

With the rate of heating employed in this experiment, then, the shadow reaction disappears at about 33,5° C. In ascertaining the lower temperature limit, holothurians were placed in beakers surrounded by vessels containing cracked ice and salt. The shadow reaction was in no case obtained below 5,0° C, but the actual limiting temperature in any one test was exceedingly difficult to place accurately.

It was found possible to abolish the shadow reflex by treating the anterior end with a weak solution of cocaine, and, incidentally to separate in this way the reactions to photic and to tactile and chemical stimuli.

Exp. 3. July 2.

4,08 P.M. Three holothurians placed in 0,5% cocaine hydrochloride for 2 minutes. Tube feet and tentacles slowly retracted, then reëxpanded. Papillae retracted. Tentacles after 1 min. gave an exceedingly faint response to shading from bright sunlight.

4,10 P.M. Returned to seawater.

4,12 P.M. Tentacles expanded. No shadow reaction perceptible. Tentacles react normally to KCl (M/10), and also to tactile stimuli, but slowly.

Therefore it is reasonable to conclude that the function of photoreception is exercised by a set of sensory elements distinct from those for mechanical and chemical reception.

The phenomena of the shadow reflex in *Holothuria* immediately invite comparison with similar reactions exhibited by sea urchins (von Uexküll 1900) serpulids, leeches, barnacles, some lamellibranchs, etc. (cf. Mast, 1911, p. 247, for review of literature). The curves of reactiveness of *Hydroids*, *Glossiphonia* (Gee, 1913), *Balanus* and *Holothuria* to repeated shadows all exhibit the characteristics of rhythmicity and rapid exhaustion. Reactions of the common Bermuda shore barnacle (*Balanus sp.*) to successive shadows are shown, for comparison, in Table IX; the reactions here consist in the retraction of the appendages and the closure of the valves; these become less complete as the stimuli succeed one another. The time taken to reopen the valves, after they had begun to close as the result of the shadow, is given to the nearest whole second. Exhaustion was more rapid at higher temperature and in brighter light.

Table IX.

The shading reflex in *Balanus*.

Exp. 4.15.2.

June 21. Retraction time, secs. Shaded at one-half minute intervals.

No. of stimulus	Diffuse light. Temp. 25,1°					Bright sunlight. Temp. 30°				
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
1	15	20	26	25	19	7	27	12	5	9
2	9	10	12	23	4	7	11	0	1	10
3	45	34	7	24	9	11	60+	0	1	14
4	2	60+	5	21	8	8	0	0	0	6
5	7	27	10	22	7	6	0	0	0	3
6	45	60+	0	38	3	7	0	0	0	1
7	0	5	0	27	26	3		0	0	0
8	0	0	0	28	60+	5		.		0
9	40	17	5	20	3	4		.		5
10	0	5	0	45	44	1		.		0
11	0	12	0	22	0	0				60+
12	0	40	0	23	0	0				0
13	5	45	0	12	0	4				0
14	0	0	0	52	0	0				0
15	0	0		0	0	2				0
16	0			0		0				

Note. When the reaction occupied more than 0,5 min., one stimulus was omitted.

In each of the above instances there is a noticeable rise in responsiveness during the first few stimulations. It is possible that this is to be accounted for (as Loeb [1912, p. 46] has explained the more rapid heliotropic movement of aphids after several trials) by the action of the products of muscular fatigue — in other words, as a treppe phenomenon. But a different partial interpretation is possible from the standpoint of a photochemical conception of light stimulation.

It is sufficiently obvious that it is not the "shadow" which stimulates, but a reduction of the intensity of the incident light.

This is shown by the facts, (1) that the rate of light reduction is a factor in the stimulus, for, to be successful in producing the reaction, shadows had to have a sharp edge, (2) that with two sources of light — e. g., a flash light and a lamp — extinguishing one of them was under certain circumstances a stimulus for the shadow reaction[1]), and (3) that the amplitude of the reaction varied directly with the intensity of the light from which the animals were shaded. No means was at hand for expressing the last relation quantitatively.

Another aspect of the shadow reaction is the converse of the familiar colored-light problem in phototropism: Is the shadow reaction initiated by the cutting out of any particular set of wave lengths? No conclusive experiments were made with *Holothuria*, but with *Balanus* it was found that cutting out either the blue or green was sufficient to produce the typical shadow reflex. The colored lights were secured by the use of glass ray filters, so the intensity relations were not clearly evident; but to the human eye the blue glass was less transparent than the red. When the barnacles had reëxpanded under the colored lights, cutting out the red or yellow produced no effect, while shading through the blue and green glasses gave typical reactions.[2]) The cutting out of the ultra-violet is certainly not a factor, as the shadow reaction was given under glass, and the sudden interposition of a glass plate between the holothurians and the sun produced no effect.

4. Discussion.

Holothuria has been shown to be photokinetic, negatively phototropic when there is a horizontal component of the incident light, and to be reactive over its whole surface to rapid decrease in light intensity. No special organs of light reception are known among the holothurians, excepting the so-called light detectors at the base of each tentacle in *Synapta hydriformis* (CLARK, 1907) and a few other species. Photic sensitivity in *Holothuria*, as in many other echinoderms, is a general integumentary function (MANGOLD, 1909, changed by light. When an animal has been for some time in sunlight which does not exceed a certain "optimum" intensity, acclimitization is brought about by the fact that the rate of photolysis of this substance and the rate of its reconstruction by the activity of the tissue cells are so related that the actual concentration of the photochemical receptor does not change appreciably, or else changes at a very slow rate. It is further assumed that either the photolytic products of this substance, or the changing concentrations of the photosensitive material itself, constitute a stimulus for nerve net processes associated with the pigment cells. If, now, the rate of photolysis be changed by increasing the intensity of the light, the acclimitization balance will be disturbed, and a stimulus thereby produced. On the other hand, if a rapid decrease in the light intensity is caused, by casting a shadow, the process of photolysis will abruptly cease, or fall to a very low rate, and the unopposed reconstruction process will initiate the shadow reflex. The concentration of the photo-sensitive material present at any given temperature and time may, for the moment, be considered as the result of a reversible reaction system

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[1]) Appropriately controlled, this method might well be used to determine the liminal stimulus at different temperatures, etc.

[2]) EWALD (1912) found that the larvae of *Balanus perforatus* were affected by sudden changes in the intensity of light, and that the green and yellow-green were the most potent in evoking these reactions as well as in effecting orientation.

$$P \rightleftharpoons a + b + \ldots$$

in which the rate of recombination of $a + b + \ldots$ in the absence of light is much greater than the rate of photolysis of P. The more rapid recombination of $a + b + \ldots$ in the absence of light, which is assumed, may be accounted for in several ways. Light may in part hinder the formation of the photosensitive material by tending to side-track some of its constituents (a, b, etc.) in other photochemical processes. Inasmuch as colloidal systems are concerned, it is almost certain that false equilibria (surface effects) are also to be reckoned with. A gross parallel of the state of affairs here pictured may be found in the migration of pigment granules in the retinular cells of crustacea and melanophores of vertebrates (cf. Parker, 1906), where the migration of the melanin is more rapid in the distal than in the proximal direction. A closer parallel is the behavior of the rod-pigment (visual purple) of vertebrates. It will likewise at once be recognized that this idea of a complex photochemical equilibrium, when more precisely developed, may be of considerable significance in the discussion of the Hering theory of color vision, the photolysis and reconstruction phases of the reaction corresponding to what psychologists refer to in the terms katabolism and anabolism.

If we agree that any change in the concentration of the photo-sensitive material may supply a stimulus for a negative reaction, in the manner above described, it is readily understood why the reaction to shading is more vigorous and more promptly carried out than is the reaction to an equivalent increase in light intensity. On this basis we may also account for the following facts:

1. Photic sensitivity is distinct from that to mechanical and chemical stimuli.
2. The sensitivity to light and to shading are together distributed over the whole surface of *Holothuria*.
3. The shadow reflex is more pronounced in bright light.
4. The shadow reflex disappears above a certain temperature, viz., the temperature above which the reconstructing reactions, already accelerated by the higher temperature, are no longer perceptibly increased in rate by shading. (The purely photolytic effects have no positive temperature coefficient.)

Increased reactiveness to shadows after the first few stimulations may be explained as a consequence of the photochemical equilibrium needing several "shocks" to get it started beyond some critical value, a sort of photochemical induction. Other features of the photic reactions of *Holothuria* may be accounted for in a similar manner.

There is some evidence that the fluorescent pigment is itself the photo-sensitive material: it absorbs the green and blue wave lengths, the part of the spectrum which is chemically the most active and which is generally the most powerful in producing phototropic orientation among animals (cf. Loeb, 1905; Gross, 1913) and in causing the shading reflex; it was found to be decomposed, slowly it is true, by light and air acting on alcohol solutions; tests of the integument of live *Holothuria* showed an abundant catalase reaction; so that it is not at all impossible that the pigment is oxidized under the influence of light in nature. The anatomical conditions are entirely favorable to this view; Polara (1906) has described and figured the situation of the pigment cells in the integument of *H. poli,* and indeed has suggested that they function as organs of light reception. These anatomical relations are as follows: in the epidermis are found scattered sense-cells, which are connected with ramifications of integumentary nerve-strands arising from the ambulacral nerves; the mesenchymal cells of the superficial connective tissue are crowded with pigment, and are connected by processes with the fibers of the integumentary nerves, and through them are brought into relation with the epidermal sense cells. I therefore believe that the yellow pigment of *Holothuria* is concerned with photo-reception.

V. Heat.

1. Introduction.

H. surinamensis is normally subjected to temperature variations of considerable amplitude occurring within relatively brief time intervals (see p. 238). It might reasonably be expected that this condition would be reflected in the behavior of *Holothuria* toward heat and heat changes. The average temperature of the sea water in which *Holothuria* was found was 28°. In connection with each laboratory experiment the temperatures of the water in aquaria in which animals were under observation was recorded; the mean of these temperatures was 27°, with a range of 22—28,5°; holothurians lived under these laboratory and temperature conditions for variable periods of two weeks and more.

In studying the effects of heat and of temperature changes on *Holothuria*, preliminary tests were made to show the influence of high and low temperatures, after which an attempt was made to discover the delicacy of the temperature sense.

2. Effects of altering the temperature.

Holothurians placed in beakers of sea water slowly heated on a sand bath were observed in ten experiments. The temperature was raised from 27° to 45° at the approximately constant rate of 1° in four minutes. Table X contains the condensed notes of these experiments.

Table X.

Exp. 49.

Temp.	Notes.
27°	Laboratory temperature at beginning of experiment.
29°	Tentacles contracted frequently.
30°	Considerable longitudinal contraction of the body. Tube feet loosened from the bottom in most cases.
31° 34°	Shadow response gradually destroyed (see page 270, 271).

33° 35°	Much peristaltic activity of the body muscles evident; waves beginning at any level run anteriorly and posteriorly with about equal frequencies. In only a few instances is more than one wave of contraction apparent at a single instant. Sensitivity to mechanical stimuli much lessened.
36°	Localized swellings begin to appear on the body wall.
38° 41°	Cloacal aperture closed by contractions of its sphincter. No reaction to mechanical stimuli by any part of the body. Animals begin to shorten the long axis, finally assuming an ellipsoidal shape. Tube feet, tentacles, etc., retracted.
41,5° 43°	Body begins to whiten underneath the pigment, owing to the coagulation of the muscle proteins. In most cases (7) this whitening began at the posterior end.
45°	Animals assume a bloated appearance, the body wall thinning out in several places, then collapsing. Dead.1) No muscular rigidity.

For producing temperatures below 27°, beakers of sea water were surrounded by cracked ice. At about 15°, the pedicels and tentacles were retracted, but in some cases the latter were re-extended. No shadow reactions were obtained below 5°. At 3° the animals had the appearance of being anaesthetised; they did not move, nor could any reactions be obtained from them; recovery from a half hour's experience of this temperature was rapid; the animals becoming normal again in about twenty minutes.

Other experiments consisted in plunging the holothurians, partially or entirely, into sea water at temperatures remote from the normal. When thus suddenly transferred to water kept at 3°, no special reaction was evident; in most cases the tentacles and pedicels slowly expanded, but reacted only very faintly to pinching with forceps, etc. Recovery on return to the normal temperature was rapid, as in the previous tests. Upon sudden transfer from 27° to water at 40°, the effect comprised vigorous general muscular contraction (in a few instances producing visceral autotomy) and the coagulation of the muscle materials, death following within five minutes. At 45° instantaneous whitening and death were the result. If merely the anterior or posterior end of an individual were thus treated, no movements were made by the animal to withdraw the affected part from the heated or cooled water. Tests for thermotaxis were then made by placing the holothurians in various posi-

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1) Frenzel (1885) states that "*Holothuria*" died in several hours at 30—40°, when suddenly subjected to that temperature.

tions along the axis of a long narrow trough in which temperature gradients were maintained by heating one end with an alcohol lamp and cooling the other with an ice-pack. No marked results were obtained, it being clear that the animals were in no sense oriented by the temperature conditions, nor did they tend to congregate more at one end of the trough than at the other.

3. Local Application of heat.

Sea water heated to 40°, 55° and 75° (the evaporation being made good with rain water) was gently pipetted in five cubic centimeter volumes over different regions of the body. In these tests the animals were placed in small dishes containing just enough water (at 26°) to cover them. Even then, however, the actual temperature of the water which reached the holothurians' surface was of course below 40°, 55°, etc. No reactions were obtained from the pedicels, papillae, or the mid-body surface; the tentacles, the anterior end and the posterior end reacted with some vigor. In none of these reactions was there any distinct bending away from the side stimulated. The responses were of about the same amplitude as those obtained with a vigorous current of sea water at the holothurians' own temperature. The tentacles occasionally reacted to currents of water at 3°. Heated glass rods and steel needles were held in close proximity to various regions of a holothurian's body when it was out of water. No constant effects were obtained. In some experiments the body wall was burned by touching with a red hot rod, but even then no clear-cut response was given.

4. Discussion.

As the result of these tests, it appears that the temperature sense, if anything properly so called is present in *Holothuria*, must be very poorly developed. The following effects of various temperatures are to be noted:

1. 2—5°. The anaesthetic-like actions of low temperatures.
2. 31—34°. The gradual cessation of the shading reactions; the intensity of the light and (possibly) the rate of heating are factors concerned.
3. 33—37°. The production of muscular peristalsis at temperatures above the normal.
4. 35—38°. The stoppage of respiratory cloacal movements by the contraction of the sphincter.
5. 38—41°. General muscular contraction.

6. 41—45°. Coagulation of the muscle substances and consequent death. "Heat rigor" is either totally absent, or passes very quickly.

7. The tentacles (especially) respond to currents of water at temperatures differing widely from the normal.

Mayer (1911, p. 125) found that, to have an animal survive in the shallow or surface waters of the tropics, it must be able to withstand a temperature of 29° C. Most of the more highly organized marine forms are capable of doing this, for (Vernon, 1899, at Naples) their death temperatures when subjected to experimental heating are in the neighborhood of 40°. Parker (1908) found the temperature producing death by coagulation to be about 40° in the case of the Bermuda *Amphioxus*. The behavior of *Holothuria* as it was gradually heated is characteristic of many other marine invertebrates, its muscle substances coagulating at about 42°, while a temperature of 45° produces almost instantaneous whitening. The maximum temperatures observed in its habitat (31,8°) were therefore well within the safety zone. The lethal temperature for some other littoral Bermuda animals would appear to be much nearer their customary thermal condition; the small translucent crustacean *Synalpheus* (inhabiting sponges), for example, was found to have its muscle proteins coagulated when slowly heated up to 37°, a temperature of only 34° causing them to become immobile.

It seems quite clear that there is no well developed capacity for the reception of thermal stimuli. Such reactions as were obtained to currents of warm sea water directed on to the tentacles are more properly interpreted as due to the lowering of the threshold to mechanical stimuli at the higher temperatures. *Thyone*, also, is "comparatively insensitive to thermic changes" (Pearse, 1908, p. 281).

VI. **Chemical stimuli.**

1. Introduction.

No quantitative analysis of the chemical sense in echinoderms has thus far been made. The experiments of Romanes (1885), Preyer (1886), Prouho (1890), Nagel (1894), Jennings (1907), v. Uexküll (1905), and others who have studied starfishes and sea urchins have demonstrated that there is present a chemical sense of the general nature of smell (i. e., a distance receptor), and also that reactions follow the direct application of dissolved electrolytes and food juices ("taste"). Both types of chemo-reception play a part in feeding (Jennings 1907, Cowles 1910). The tube feet are the most sensitive organs (Prouho 1890, Hamann 1883, Cuénot 1891, Cowles 1910). The oral tentacles of ophiuroids were believed by Preyer (1886) and Nagel (1894) to be sensitive also (cf. likewise v. Uexküll, 1905). Synaptids have been thought to have some chemical sense associated with the so-called olfactory cups on the tentacles (Clark 1901a).

Pearse (1908, p. 275) found *Thyone* to react negatively to supposed food extracts; this, together with Nagel's brief statement on *Cucumaria*,— „Diese überaus trägen Tiere zeigen jedoch vollkommene Gleichgiltigkeit gegen alle von mir angewandten süssen und bitteren Reizstoffe" (Nagel, 1894, p. 178),— and a few experiments of Polara (1906) are the only observations known to me on the reactions of holothurians to chemical stimuli.

The normal feeding of *H. surinamensis* probably is not especially concerned with chemical sensitivity; it is more likely merely a complex of tactile responses, as seems to be the case with many synaptids. The reactions obtained to dissolved substances were of a strictly negative kind, and I have therefore not dealt with this phase of the problem.

Experiments were made with the object of demonstrating the range of substances to which *Holothuria* is sensitive, the distribution of this sensitivity over the body, and such quantitative aspects of the reactions as would permit of comparison with the physiology of chemo-reception in other animals.

2. Method; reactions obtained.

Qualitative experiments in which the stimulus was applied to various parts of the animals in the form of solutions, or, in the case of the essential oils, used directly, showed that all portions of the surface of *Holothuria* were sensitive to salts, acids, alkalies, alkaloids, certain carbohydrates, various other organic substances, and essential oils. All the reactions, with one exception, were of the negative kind previously described under the reactions to mechanical and photic stimuli, tending toward the removal of the part affected from the field of stimulation. The one exception was in the case of magnesium chloride in M/10 solution; the tube feet did not react negatively, but in most cases extended and frequently bent toward the stream of solution coming from a pipette. Tannic acid, potassium hydroxide, and digatilin, in fairly concentrated solutions, produced negative reactions of a type not found in connection with any other stimulus. In one curious reflex the anterior and posterior ends were bent slightly toward each other, and the animal rolled over completely, away from the side stimulated. Tannic acid (M/10) stimulated the posterior end so strongly as to cause it to be turned from side to side for three or four times. Hydrogen peroxide in 3% solution (approximately M/1), and in dilutions down to 0,75%, stimulated all parts of *Holothuria's* surface so long as bubbles of oxygen appeared on the part bathed by the solution. This test indicates the presence of catalase in the surface integument of *Holothuria*, which I believe may be concerned in the production, or destruction, of pigment materials. The solution of H_2O_2 used contained a small amount of acetanilid (as a preservative), but when the H_2O_2 had been destroyed by boiling, and the solution reaërated, no reactions were given to it; therefore the acetanilid (not destroyed by boiling) was not concerned in the stimulation.

The effects of sufficiently intense stimuli were rapidly carried anteriorly and posteriorly from points in the mid-region; a good instance of this was seen in the effect of tannic acid solutions, where the anterior and posterior ends of the animals would close up and turn away from the side stimulated before the appearance of an insinking of the body-wall at the point of stimulation.

No new facts were brought to light by experiments with mutilated animals. As with tactile and photic stimuli, reactiveness was lower in holothurians with the nerve ring and its associated structures amputated, the region nearest the cut being especially insensitive.

The very slightly soluble materials used were: ethyl ether, chloroform, xylol, clove oil, oil of Bergamot and oil of origanum. Tentacles, brim, and cloacal termination reacted to drops of these substances held at the tip of a pipette at a distance of two centimeters from them. The pedicels, papillae, and body surface reacted only when actually touched by a drop, sharp reactions then being given. Sea water which has stood in contact with these substances also produced a negative reaction. In all cases the order of reactiveness of the several parts of the animal was the same as that which has already been found for tactile and photic stimuli, namely: tentacles > anterior end > posterior end > podia > mid-body surface.

3. Quantitative experiments.

For tests designed to be on a more exact basis, the following method was employed:

Solutions of the materials to be used as stimulants were made up on a gram-molecular basis at 25°, usually M/10, with filtered rain water. The stimulus was applied by allowing 0,5 cc to flow gently over the desired region from a pipette the tip of which was held one-half centimeter away from the point to be stimulated. The reaction time was measured (with a stop watch reading to 0,1 sec.) from the time of application of the stimulus until some movement was given by the animal. The tests were made in diffuse light, to avoid shadow stimuli. The rain water used was tasteless, neutral to indicators, and in sunlight algae grew in it (so it was not toxic in this sense); analysis showed the presence of minute amounts of calcium chlorides and sulphates, such impurities being expected, as the water was collected from whitewashed rain sheds. For the purpose of the present experiments, it was essentially "distilled water." The chemicals employed were all Merck's materials of high purity. Many careful tests showed that under the conditions of these experiments rain water in itself did not constitute a stimulus. *Holothuria* did not react to solutions of merely high or low osmotic pressure — a condition which is, I believe, correlated with the fact that, living as they do near low-tide level, these animals must frequently be exposed to dilution of the sea water by heavy downpours of tropical rain. Henri et Lalou (1903) found the exposed membranes of *Holothuria* and *Stichopus* to be efficient semipermeable membranes, permitting the osmotic regulation of the internal fluids in a fairly rapid manner. In my experiments rapid death, however, resulted from sudden immersion in rain water, as shown by the following:

Exp. 82.

July 20. Temp. 25,1°.

A.M.	Elapsed time (minutes)	
10,30		Four holothurians plunged into 1 liter of rain water.
10,31	1	Tentacles and podia retracted. Green pigment becoming dissolved out.
10,55	25	One animal dead; another has begun to eviscerate. Further pigment loss.

11,20	50	One other animal eviscerating; considerable longitudinal folding of the body wall in all. First evisceration complete.
11,40	70	Two more dead.
11,55	85	All dead, in the extended state, with the tube feet, etc., withdrawn.

One noteworthy result of Exp. 82 is the rapid solution of the integumentary fluorescent pigment by fresh water; this effect would form a very convenient indicator in studies of permeability changes.

Mixtures of sea water and rain water in the proportion of 1 : 1 gave no reactions. Sea water concentrated to 0,5 its original volume [1]) and reaërated by splashing did produce reactions from all parts of *Holothuria* save the tube feet and papillae. The reactions were of very slight amplitude, and I believe are to be accounted for, not by the increased osmotic pressure of the stimulant, but rather by the disturbance of the normal electrolyte balance in sea water with changing concentration; Spaeth (1913, p. 540) found that diluted sea water stimulated *Fundulus* melanophores through the preponderance of the sodium-ion. Another source of error in these measurements arises from the fact that the water currents from the pipette might also serve as a tactile stimulus. Repeated treatment with M/10 KCl, for example, so sensitized *Holothuria's* integument that it did react to currents of fresh water, and even to those of sea water. Jennings (1907, p. 69) found a similar interrelation of chemical and tactile reactions in the pedicellaria rosettes of *Asterias forreri*. Accordingly, a sufficient period of rest (five minutes) was allowed between successive stimulations, and frequent control tests were made with the rain water alone. Tables XIII to XIX, inclusive, summarize the results of these experiments. Each of the reaction-time figures is the mean of from ten to fifteen measurements made on three to five animals, the agreement between the individual measurements being sufficiently close to warrant the use of this comparatively small number of measurements and animals for the purpose of a general investigation. The several random examples given in Tables XI and XII will serve to emphasize this point. A few other substances tested qualitatively will be referred to in the discussion.

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1) Bermuda sea water normally contains about 36,5 parts of salt per 1000 (Mark, 1913).

Table XI — K_2SO_4 M/20.

Exp. 97.

Part Stimulated	Reaction time in seconds										Mean
Tentacles	1,0	1,0	0,6	0,8	0,6	0,5	1,0	0,7	0,8	1,0	0,80
Middle	2,0	1,7	2,7	2,0	2,4	2,0	2,0	2,0	2,3	1,8	2,09
Posterior end	1,2	2,6	1,8	1,8	1,0	1,2	1,4	1,6	2,4	1,2	1,62
Podia	1,8	1,8	1,2	1,2	1,2	1,6	1,0	1,5	1,4	1,9	1,46

Table XII. — KCl M/10.

Exp. 15.1.

Part Stimulated	Reaction time in seconds										Mean
Tentacles	0,4	0,9	0,5	0,4	0,4	0,6	0,4	0,2	0,6	0,4	0,48
Middle	2,2	3,4	2,8	4,2	3,8	2,0	3,4	3,0	5,0	3,2	3,20
Posterior end	3,0	0,8	2,0	0,6	1,0	1,5	0,8	0,8	0,6	0,8	1,19
Podia	1,2	0,8	1,0	1,2	0,6	0,4	0,6	0,6	0,4	0,8	0,76

Note. In Tables XIII—XIX reaction time is in seconds.

Table XIII. — Salts N/10.

Part tested	KCl	NaCl	LiCl	K_2CrO_4	NH_4Cl	K_2SO_4	$MgCl_2$
Tentacles	0,48	0,60	0,81	0,80	0,80	0,80	1,8
Middle	3,2	1,7	2,5	2,1	5,6	2,1	9,5 1)
Posterior end	1,2	1,0	1,1	1,4	2,2	1,6	1,2
Podia	0,76	1,6	1,8	1,4	2,3	1,5	∞

1) Many "no reaction" cases.

Table XIV. — Potassium chloride.

Part tested	Molecular dilution						
	1	10	50	100	250	500	1000
Tentacles	0,44	0,48	0,82	0,68	0,80	1,95	∞ 1)
Middle	1,2	3,2	5,4	7,3	0,68	5,1 2)	∞
Posterior end	0,56	1,2	1,7	0,93	1,80	3,0	∞
Podia	0,56	0,76	0,82	1,29	1,4	3,6 2)	∞

1) ∞ = "no reaction". 2) Several ∞ cases.

Table XV. — Acids M/10.

Part tested	Hydrochloric	Acetic	Lactic	Malic	Tannic
Tentacles	0,20	0,31	0,85	0,40	0,65
Middle	1,1	1,4	1,9	1,9	1,4
Posterior end	0,50	0,80	0,91	0,35	1,1
Podia	0,61	0,53	0,90	0,71	0,92

Table XVI.— Hydrochloric Acid.

Part tested	Molecular dilution				
	10	50	250	500	1000
Tentacles	0,20	0,41	0,79	0,69	∞
Middle	1,1	1,6	2,0	2,3 1)	∞
Posterior end	0,50	1,2	0,83	1,1	∞
Podia	0,61	0,56	0,96	1,2	∞

1) Several "no reaction" cases.

Table XVII.— Acetic Acid.

Part tested	Molecular dilution				
	10	50	100	500	1000
Tentacles	0,31	0,77	1,2	1,8	∞ ?
Middle	1,4	1,6	1,9	4,6 1)	∞
Posterior end	0,80	1,3	2,0	2,6	∞
Podia	0,53	0,61	0,92	1,7	∞

1) Several "no reaction" cases.

Table XVIII.— Potassium Hydroxide.

Part tested	Molecular dilution			
	10	100	500	1000
Tentacles	0,30	0,35	0,55	∞
Middle	1,4	1,4	1,5	∞
Posterior end	0,53	0,96	1,5	∞
Podia	0,96	1,2	0,80	∞

Table XIX.— Other Substances.

Substance	Maltose		Cane Sugar		Glycerin		Atropine Sulphate	Acetamid		
Concentr.	M/10	M/50	2 M	M/10	100%	M/10	M/100	M/2	M/5	M/50
Tentacles	1,4	∞	∞	∞	0,50	0,67	0,46	1,1	3,9	∞
Middle	5,2	∞	∞	∞	5,0	∞	2,0	2,4	4,2	∞
Posterior end	1,9	∞	∞	∞	1,2	4,8	1,4	1,6	3,5	∞
Podia	2,7	∞	∞	∞	0,90	∞	0,84	1,6	∞	∞

4. Discussion.

From the standpoint of the present experiments there are two aspects of reaction to chemical stimuli — 1. the reaction time, and 2. the extent or amplitude of the effect. Reaction-time measurements take into account only one of these factors, while for the other there is no convenient method of quantitative expression. In general, they paralleled each other, a brief reaction time being associated with a reflex of wide amplitude, but this was by no means an invariable rule. For example, comparing the reactions of the anterior and posterior ends to M/5 acetamid (Table XIX), the mean reaction time of the tentacles (3,9 sec.) was slightly greater than that of the posterior end (3,5), but the amplitude was much greater with the anterior end. Taking all the aspects of the reactions into consideration, the order of reactiveness of the various parts of *Holothuria* is, however, the same whether reaction time or amplitude be made the criterion, and is as follows: tentacles > anterior end > posterior end > tube-feet, papillae > mid-body surface.

It was difficult to stimulate the anterior part of the surface of *Holothuria* without involving the tentacles, but the following experiment with M/10 NH_4Cl will show the relative sensitivity of the anterior end:

a) Tentacles expanded; reaction time 0,8 sec.
b) Tentacles retracted (i. e. not stimulated).; reaction time 1.8 sec.

Five observations were made on each condition, in the same individual.

Considering (1) the reaction-time, (2) the amplitude of the effect, and (3) the limiting concentrations effective as stimuli, for all the parts stimulated, the order of decreasing stimulus intensity of representative substances was: —

hydrochloric acid > atropine sulphate > acetic acid > potassium hydroxide > potassium chloride > maltose > acetamid > glycerin.

Within the several groups of substances studied, the relative efficiency of stimulation, at the concentrations noted, was: —

Acids (Table XV),
hydrochloric > acetic > (picric) > tannic > malic > lactic (M/10).

Salts (Table XIII),
potassium chloride > sodium chloride > lithium chloride > pot-

assium chromate>ammonium chloride>potassium sulphate> magnesium chloride (N/10).

Carbohydrates (Table XIX),

maltose>(dextrose) cane sugar, (M/10).

Alkaloids (Table XIX),

atropine sulphate > strychnine sulphate > quinine hydrochloride (M/100).

Holothuria is reactive to substances which on the human tongue give rise to the four distinct taste qualities, — salt, sour, bitter, sweet, — and reacts also to potassium hydroxide, representing the "alkaline taste". The case of the sweet substances is especially interesting. PARKER (1912, p. 228) believes that aquatic vertebrates are insensitive to stimulation by sugars and the like, and that the acquisition of such sensitivity by higher forms is possibly associated with the storage of carbohydrates in terrestrial vegetation. His experiments, and those of SHELDON (1909) on the dogfish which gave the same result, were made only with cane sugar (and, in SHELDON'S experiments, dextrose). I found that *Holothuria* was likewise insensitive to cane sugar even in saturated solutions. The same was true of dextrose. But maltose in M/10 solution gave good reactions, as did glycerin also. I therefore believe that fishes and other aquatic animals may very probably be found reactive to sugars and other "sweet" substances when a larger range of "sweet" stimulants has been investigated.

The lack of reaction to rain water and to saturated cane-sugar solutions is sufficient evidence that the stimulus by dissolved substances is not osmotic. It is significant that the limiting stimulation-concentrations of HCl (M/500+), KOH (M/500), KCl (M/500+), acetic acid (M/500+), and maltose (M/10) are of the order of magnitude which we associate with taste. Evidence from the electrolytes used shows that both anions and kations play a rôle in the stimulation process. The reaction-time measurements with the chlorides, for example, give series which vary somewhat with the part of the animal considered, but taking the tentacles alone the order is (Table XIII):

$$K>Na>Li,\ NH_4>Mg$$

and the three salts of potassium used give, for the same part, the series:

$$Cl>SO_4,\ CrO_4\ (M/10)$$

The tables of reaction times also show peculiarities which have been recognized in the chemical stimulation of other animals. Comparing the effects of hydrochloric and acetic acids, it will be seen that the acetic acid has a far higher stimulating power than would be predicted from its dissociation, on the assumption that the free hydrogen ion alone is concerned. This is likewise true of all the other organic acids, and is comparable to the effect in man (Richards 1898) and in the earthworm (Hurwitz 1910). There is, I believe, a possibility that *Holothuria's* muscles are stimulated directly by such irritants as tannic acid, etc. One other point in the reaction-time results is the greater stimulating effect of potassium chloride (Table XIV) in dilute solutions. The relation of concentration to stimulating effect was studied especially with potassium chloride, hydrochloric acid, acetic acid, and potassium hydroxide. In general, reaction time increases with decreasing concentration, but with (Table XIV) potassium chloride and (Table XVI) hydrochloric acid there is a quite perceptible decrease in reaction time in the neighborhood of $\frac{M}{100} - \frac{M}{250}$, except in the case of the podia. This drop in reaction-time was accompanied by an increase in the amplitude of reaction, and was therefore a real phenomenon, paralleled by the results of Parker and Metcalf (1906) on the earthworm.

It has been shown in this paper that the reaction to chemical stimuli is a general integumentary function in *Holothuria*, though the exact chemo-receptors have not been anatomically distinguished. In all probability, however, they are of the usual invertebrate type, comparable with the chemo-receptors of the earthworm (cf. Bovard 1904) and the olfactory cells of vertebrates. According to a conception of the evolution of organs of chemical sense in vertebrates which has been developed by Parker (1912), the end organs of smell, the common chemical sense, and of taste are related in a genetic series, of which the olfactory receptor is phylogenetically the oldest. Now, if we may legitimately run this series back to its invertebrate source, several things might reasonably be predicted, namely, 1) that the olfactory epithelium of some lower vertebrates would retain traces of its sensitivity to dissolved materials which ordinarily constitute homologous stimuli for taste, and 2) that the chemo-receptors of invertebrates would show, with reference to the range of concentrations which we associate with taste, a mechanism of stimulation different in some essential particulars from that exhibited by vertebrate taste buds, for the latter are receptor cells in a secondary sense only. If SCOTT's (1913, p. 31) incidental observation that the dogfish *Mustelus canis* fails to react to fresh water when the nostrils are plugged be substantiated, it would appear that the first possibility is actually realized. Concerning the second expectation, the evidence from the earthworm (PARKER AND METCALF, 1906) and that from *Holothuria* are in agreement, to the effect that with the chlorides the kation effect predominates over that of the anion, whereas with the human salt-taste the chlorine ion is the principal stimulating agent. Stimulation by dissolved materials is a process which involves certain fundamental properties of the receptive mechanism as well as of the stimulant, so that such similarities as there are between taste in vertebrates and in invertebrates may readily be accounted for.

VII. **Summary.**

I.

(1) *Holothuria surinamensis* LUDWIG is reactive to tactile, vibratory, photic and chemical stimuli. It is practically indifferent, in a sensory way, to heat.

(2) To all stimuli the order of decreasing sensitivity of the parts of the body is:

tentacles > anterior end > posterior end > papillae, pedicels (podia) > mid-body surface.

(3) The tube-feet discs are positively stereotropic. This is responsible for the righting reaction.

(4) *Holothuria* is not geotropic. The climbing of vertical walls is due to the persistence of an impulse to movement.

(5) *Holothuria* is photokinetic, negatively phototropic, and gives a negative reaction to sudden decrease in light intensity; it does not react to an increase in light intensity. The whole surface is sensitive to photic stimuli.

(6) These photic reactions are explainable on the basis of a photochemical conception of the action of light. The fluorescent integumentary pigment is possibly concerned in this matter.

(7) Dissolved substances representing homologous stimuli for the human taste qualities — sour, bitter, sweet, salt (and "alkaline") are effective as stimuli.

(8) The details of chemical stimulation are parallel to those in

the earthworm, and support the view that the physiology of "taste" in vertebrates is different in essential particulars from that in invertebrates.

II.

The present paper has been devoted to the description and interpretation of a series of individual reactions. It must be emphasized, however, that both in the laboratory and under the natural conditions of life these separate reactions of *Holothuria* are welded into an harmonious behavior, which is closely correlated with the animal's passively defensive mode of existence. Morphologically the holothurians are set apart from other echinoderms by the immense development of their muscle equipment and the concomitant reduction of the calcareous exoskeleton. It is this prominence of the muscular system which gives to the behavior of *Holothuria* a kind of unity not seen, so conspicuously at least, in other echinoderms. As far as its behavior is concerned *Holothuria surinamensis* is a bilateral animal; the restriction of pedicels to its functionally ventral surface helps to accentuate this. Its direction of progression is definitely fixed; it always moves with the anterior end in advance. The tentacles, pedicels and papillae are, to a certain extent, independently controlled; but as a whole the flexibility of the animal and the possibility of coördination, even in the absence of the nerve ring, makes *Holothuria* much less a "republic of reflexes" (v. UEXKÜLL) than is the case with the sea-urchin or starfish.

Bibliography.

BANCROFT, F. W., 1913, Heliotropism, differential sensibility, and galvanotropism in Euglena, in: Journ. exper. Zool., Vol. 15, No. 4, p. 383—428.

BECHER, S., 1909, Die Hörbläschen der Leptosynapta bergensis. Ein Beitrag zur Kenntnis der statischen Organe, in: Biol. Ctrbl., Vol. 29, p. 413—425.

BIEDERMANN, W., 1889, Zur Physiologie der glatten Muskeln, in: Arch. ges. Physiol., Vol. 46, p. 398—421.

BOVARD, J. F., 1904, The distribution of the sense cells in Microscolex elegans, in: Univ. California Publ., Zool., Vol. 1, No. 8, p. 269—286, 2 pls.

BRIOT, A., 1906a, Sur les corps bruns des holothuries, in: C.R. Soc. Biol. Paris, Vol. 60, p. 1156—1157.

—, 1906b, Différenciation physiologique des diverses espèces d'Holothuria, ibid., p. 1157—1158.

CHESTER, W. M., 1912, Wound closure and polarity in the tentacle of Metridium marginatum, in: Journ. exper. Zool., Vol. 13, No. 3, p. 451—470.

CLARK, H. L., 1898. Notes on the echinoderms of Bermuda, in: Ann. New York Acad. Sc., Vol. 11, p. 407—413.

—, 1899, Further notes on the echinoderms of Bermuda, ibid., Vol. 12, p. 117—138.

—, 1901a, The Synaptas of the New England coast, in: Bull. U. S. Fish Comm., Vol. 19 (1899), p. 21—31, 2 pls.

—, 1901b, Bermudan echinoderms, in: Proc. Boston Soc. nat. Hist., Vol. 29, No. 16, p. 339—345.

—, 1901c, The echinoderms of Porto Rico, in: Bull. U. S. Fish Comm., Vol. 20 (1900), Pt. 2, p. 231—263, 4 pls.

Loeb, J., 1891, Ueber Geotropismus bei Thieren, in: Arch. ges. Physiol., Vol. 49, p. 175—189.

—, 1893, Ueber künstliche Umwandlung positiv heliotropischer Thiere in negativ heliotropische und umgekehrt, ibid., Vol. 54, p. 81—107.

—, 1905, Studies in general physiology, Pt. 1, xiii + 423 pp., 8vo., Chicago.

—, 1912, The mechanistic conception of life, 232 pp., 12 mo, Chicago.

Ludwig, H., 1875, Beiträge zur Kenntnis der Holothurien, in: Arb. zool.-zoot. Inst. Würzburg, Vol. 2, p. 77—118.

MacCurdy, H. M., 1912, Observations on the reactions of Asterias forbesii to light, in: Science (N.S.), Vol. 35, p. 192.

MacMunn, C. A., 1889, Contributions to animal chromatology, in: Quart. Journ. microsc. Sc., Vol. 30, No. 118 (N.S.), p. 51—96, tab. 6.

Mangold, E., 1909, Sinnesphysiologische Studien an Echinodermen. Ihre Reaktionen auf Licht und Schatten und die negative Geotaxis bei Asterina, in: Ztschr. allg. Physiol., Vol. 9, p. 112—146.

Mark, K. L., 1913, Preliminary study of the salinity of sea water in the Bermudas, in: Proc. Amer. Acad. Arts Sc., Vol. 48, No. 18, p. 671—678.

Mast, S. O., 1911, Light and the behavior of organisms, x + 410 pp., 12 mo., New York.

Mayer, A. G., 1911, Annual report of the Director, Dept. of marine Biol., in: Carnegie Inst., Year Book No. 10, p. 110—156, tab. 3—6.

Mitsukuri, K., 1903, Notes on the habits and life history of Stichopus japonicus Selenka, in: Annot. zool. Japon., Vol. 5, pt. 1, p. 1—21.

Moore, A. R., 1910, On the righting movements of the starfish, in: Biol. Bull., Vol. 19, No. 4, p. 235—239.

Moore, B., 1913, [Report], in: The marine biological station at Port Erin, in: Proc. Trans. Liverpool biol. Soc., Vol. 27, p. 49.

Nagel, W. A., 1894, Vergleichende physiologische und anatomische Untersuchungen über den Geruchs- und Geschmackssinn und ihre Organe, in: Bibliotheca zool., Vol. 7, Heft 18, viii + 207 pp., 7 Taf.

Parker, G. H., 1906, The influence of light and heat on the movement of the melanophore pigment, especially in lizards, in: Journ. exper. Zool., Vol. 3, No. 3, p. 401—414.

—, 1908, The sensory reactions of Amphioxus, in: Proc. Amer. Acad. Arts Sc., Vol. 43, No. 16, p. 415—455.

—, 1911, The mechanism of locomotion in gasteropods, in: Journ. Morphol., Vol. 22, No. 1, p. 155—170.

—, 1912, The relation of smell, taste, and the common chemical sense in vertebrates, in: Journ. Acad. nat. Sc. Philadelphia (2), Vol. 15, p. 221-234.

Parker, G. H. and C. R. Metcalf, 1906, The reactions of earthworms to salts, in: Amer. Journ. Physiol., Vol. 17, No. 1, p. 55-74.

Pearse, A. S., 1908, Observations on the behavior of the Holothurian, Thyone briareus (Lesueur), in: Biol. Bull., Vol. 15, No. 6, p. 259—288.

—, 1909, Autotomy in Holothurians, ibid., Vol. 18, No. 1, p. 42—49.

POLARA, G., 1906a, Sulla connessione delle cellule pigmentate del mesenchima cutaneo delle Oloturie con i nervi cutanei e sul loro significato funzionale (Nota preliminare), in: Boll. Accad. Gioenia Catania, Fasc. 88, p. 12—24.

—, 1906b, Sopra alcuni speciali corpuscoli di senso delle Oloturie, ibid., Fasc. 90, p. 21—28.

PREYER, W., 1886, Ueber die Bewegungen der Seesterne, in: Mitth. zool. Stat. Neapel, Vol. 7, p. 27—127; Vol. 8, p. 191—223.

PROUHO, H., 1890, Du sens de l'odorat chez les étoiles de mer, in: C.R. Acad. Sc. Paris, Vol. 110, p. 1343—1346.

RAND, H. W., 1909, Wound reparation and polarity in the tentacles of Actinians, in: Journ. exper. Zool., Vol. 7, No. 2, p. 189—238, 2 pls.

RETZIUS, G., 1906, Über die Verteilungen der Sinnesnervenzellen in der Haut der Holothurien, in: Biol. Unters. (2), Vol. 13, No. 14, p. 113—116.

RICHARDS, T. W., 1898, The relation of the taste of acids to their degree of dissociation, in: Amer. chem. Journ., Vol. 20, p. 121—126.

ROMANES, G. J., 1885, Jellyfish, starfish, and sea urchins, in: Internat. sc. Ser. VII + 323 pp., New York.

SEMPER, C., 1868, Reisen im Archipel der Philippinen. II. Wissenschaftliche Resultate, Vol. 1, Holothurien, IV + 888 pp., 40 pls., Leipzig.

SCOTT, G. G., 1913, A physiological study of the changes in Mustelus canis produced by modifications in the molecular concentration of the external medium, in: Ann. New York Acad. Sc., Vol. 23, p. 1—75.

SHELDON, R. S., 1909, The reactions of the dogfish to chemical stimuli, in: Journ. comp. Neurol., Vol. 19, No. 3, p. 273—311.

SPAETH, R. A., 1913, The physiology of the chromatophores of fishes, in: Journ. exper. Zool., Vol. 15, No. 4, p. 527—585, 4 pls.

TORELLE, E., 1909, Regeneration in Holothuria, in: Zool. Anz., Vol. 35, p. 15—22.

UEXKÜLL, J. v., 1896, Zur Muskel- und Nervenphysiologie des Sipunculus nudus, in: Ztschr. Biol., Vol. 33, p. 1—27.

—, 1900a, Die Physiologie des Seeigelstachels, ibid., Vol. 39, p. 73—112.

—, 1900b, Die Wirkung von Licht und Schatten auf die Seeigel, ibid., Vol. 40, p. 447—476.

—, 1904, Studien über den Tonus. II. Die Bewegungen der Schlangensterne, ibid., Vol. 46, p. 1—37, tab. 1—2.

VERNON, H. M., 1899, The death temperatures of certain marine organisms, in: Journ. Physiol., Vol. 25, p. 131—138.

VERRILL, A. E., 1906, The Bermuda Islands. Part V.— Characteristic life of the Bermuda coral reefs, in: Trans. Connecticut Acad. Arts Sc., Vol. 12, p. 204—348 (index, p. 413—418), 22 pls. (tab. 28—40).

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting. *Separate:* 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773, 4 pls. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569–626, 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zoöl. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 pp. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zoöl. Jahrb., Abt. f. Physiol., **35** (3) : 233–297. Aug., 1915.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1

1. Cole, L. J.—Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A.—The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H.—A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R.—The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B.—The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L.—The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T.—Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W.—The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D.—The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. Blackman, M. W.—The Spermatogenesis of tne Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. Linton, E.—Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H.—The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. Cole, J. F.—Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S.—The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. Morgulis, S.—Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. Rand, H. W.—Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. Mark, E. L.—The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. Smallwood, W. M.—Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G.—Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W.—Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O.—Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. Cowdry, E. V.—The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. Gross, A. O.—Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M.—Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773, 4 pls. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569–626, 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zoöl. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 pp. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zoöl. Jahrb., Abt. f. Physiol., **35** (3) : 233–297. Aug., 1915.

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No. 34.

THE ORIENTATION OF A HOLOTHURIAN BY LIGHT.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.
NO. 255.

THE ORIENTATION OF A HOLOTHURIAN BY LIGHT

W. J. CROZIER[1]

Received for publication October 8, 1914

I. In a paper dealing with the sensory reactions of Holothuria surinamensis, which is to appear in the Zoologischer Jahrbücher, I have shown (Crozier, 1914 [?]) that the analysis of echinoderm reactions to light given by Mast (1911, pp. 211–213) fails when applied to Aspidochirote holothurians, since (1) these animals move only with the anterior end in advance, and (2) they *orient away* from the light, yet react *negatively* to shading. It may not be out of place to publish a more detailed account of the photic orientation of another holothurian, H. captiva, which exhibits this reaction in an even more clearly defined manner.

This study was carried out at the Bermuda Biological Station for Research during the summer of 1914. For the use of the facilities of the station, my best thanks are due the Director, Prof. E. L. Mark.

II. Holothuria captiva Ludwig is a small dark-green Aspidochirote holothurian,[2] which is relatively common in certain localities in the Bermudas. It is found exclusively, during the daytime at least, clinging to the under sides of slabs of stone on rather exposed shores, just under low water mark. Individuals ranging in length from 4 to 100 mm. are obtainable during the months of June and July.[3] Experiments were made upon animals covering this range in size.

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[1] Contributions from the Bermuda Biological Station for Research. No. 34.

[2] The paper on H. surinamensis contains general observations on the behavior of H. captiva.

[3] The majority of the younger specimens were found at the entrance to Hungry Bay.

The bilateral symmetry of this form is more strongly pronounced than is the case with the related H. surinamensis and H. rathbuni, though, as might be expected from the near structural affinity of all three, they show many points in common. The accentuation of physiological polarization in H. captiva is due mainly to the fact that the ventral trivium (which alone bears tube feet) is, even in very young animals, more flattened and sharply marked off from the lateral body surfaces than in the other two species; a minor factor is the greater obliquity of the oral plane, which runs obliquely ventrad and posteriad. This bilaterality is further brought out by the relatively greater rigidity of the body; lengthwise spiral twisting, common in H. surinamensis, does not occur here, and the main tendency of the body, in the absence of special stimuli, is to preserve the straightness of its horizontal axis.

The anus is situated dorsally, and when the animal is intensely stimulated, the brim about it may be elevated into a chimney-like tube, capable of being directed from side to side. This is connected with the great development of Cuvierian organs in this species, for the tube, being directed toward an irritated point on the body, controls in a general way the direction in which these organs are discharged.

In the youngest stages found (4 to 10 mm. long) the pigmentation of the body differs from that of the larger specimens, since in the very small animals the whole body is of a light green color, while in the older ones this hue persists only on the trivium, in the tentacles, and on the tips of the dorsal papillae, the dorsal and lateral surfaces becoming deep olive green. Even in the youngest specimens the tentacles are less highly colored than is the rest of the body. Darker pigmentation first becomes evident about the bases of the papillae, in animals about 8–9 mm. long; it is due, largely, to an increase in the amount of the lighter pigment, though another substance, dark brown and chemically distinct from the former, is also concerned. Some chemical characteristics of the green pigment and its possible rôle in photoreception have been treated of in the paper above referred to (Crozier, 1914 [?]), and will be further considered below.

III. Holothuria captiva ordinarily lives in dark situations. To keep it in a healthy condition for any length of time in the laboratory, it must be shaded. Prolonged exposure to even moderately bright diffused light exerts a distinctly toxic effect. It is photokinetic. The whole surface of the animal is sensitive to light. Tests made with the aid of an apparatus designed to produce small areas of light[4] gave results entirely consistent with those previously found for H. surinamensis. The tentacles reacted negatively, as shown by their partial contraction and by more or less undirected waving movements. The pedicels (when the spot-light was intense) became detached and waved about. The papillae were caused to collapse. Every point on the body-surface reacted negatively to sufficiently strong light by local in-sinking.[5] The order of sensitivity of the parts of the animal is: anterior end > posterior end > pedicels, papillae > mid-body surface.

The method of orientation to horizontal light of not too great an intensity, coming from a single source, will be apparent from an examination of the sample trails given in the text figures and in the plate. The tests, with the exception of those shown, were made by admitting sunlight, or light from a 40 c.p. tungsten filament, through a diaphram into a blackened box containing the holothurians in a flat-sided glass aquarium. Sketches of the moving animals were secured, in a dark-room, by having the aquaria mounted on short legs above a table, so that outlines of their successive positions during orientation could be conveniently traced on paper placed beneath them. The photographs were made with the holothurians exposed to two sources of light, diffuse daylight and more intense sunlight reflected horizontally by a mirror. The movements of orientation are sufficiently slow to permit the use of an ordinary "kodak," clamped to an upright for obtaining records. No differences in the mechanism of orientation were noticeable under these slightly different experimental conditions.

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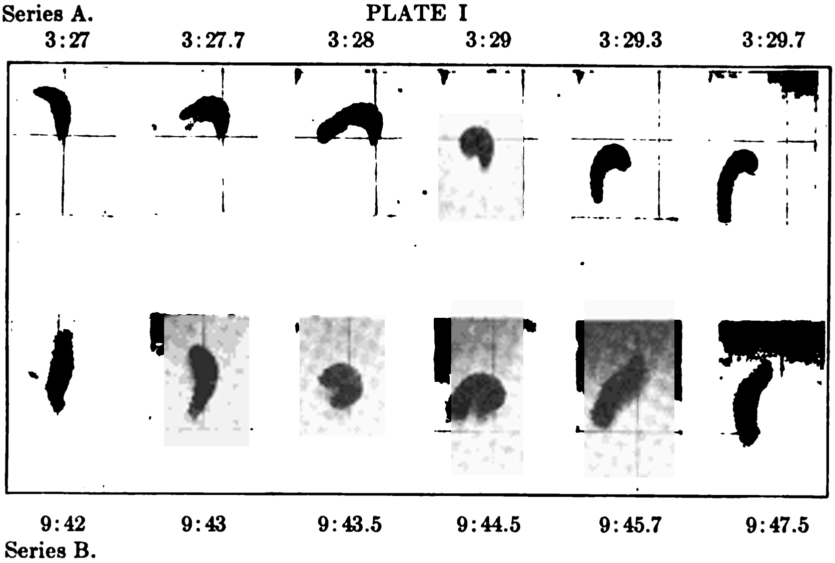
[4] This apparatus, modified from one devised by Dr. B. M. Patten, consists essentially of a small pocket electric flash-light with a tungsten filament mounted, in place of the ocular, on a microscope tube provided with a low power objective (A* or "3"). Diaphrams are readily adjusted between the electric bulb and the objective, and if necessary within the lens-system, in such a way as to give beams of light of any desired cross-section and devoid of halos. The instrument as thus constructed is self contained and convenient to handle.

[5] There is no question of a heat effect being involved, since I have found that Holothuria is not equipped with anything which might properly be termed a temperature sense.

If H. captiva is suddenly subjected to horizontal light parallel to its long axis thrown on its anterior end, the pedicels of that region are released and the anterior half of the body is swung sharply to one side (Plate I; and figs. 1, 2, 3). The swinging movement continues until the anterior end is turned as far away from the light as possible; the anterior end is then extended somewhat and re-attached; the posterior end is then frequently drawn forward by contraction of the anterior body muscles (Plate I,

PLATE I



EXPLANATION OF PLATE

Stages in the orientation of Holothuria captiva by sunlight parallel to its long axis incident upon its anterior end. In Series A moderately bright light was used, in Series B, light of lower intensity. The crossed lines shown were on a card placed beneath the aquarium. The rate of orientation can be judged by the relation of the animal to these coördinates at the times indicated opposite each picture.

figs. 2, 3); the turning away from the light continues until the animal is finally crawling in the direction of the beam. Its rate of locomotion then decreases, for the area exposed to the light is lessened; moreover, the sensitivity of the posterior end is less than that of the anterior one. Similar movements follow the application of light to the side of the animal (fig. 4). In every case the first movement is away from the light. It is only occasionally that anything which might, by any stretch of the imagination, be termed a trial movement appears. One such case is here recorded (fig. 2). It is readily accounted for by the tendency to persistence which echinoderm movements in general display. The first turning movement was carried too far (3:35), and stimulation on the other side forced the anterior end to swing back; it is to be noted that these pseudo "trial movements" are not maintained.

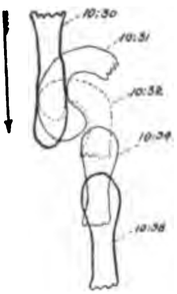


FIG. 1

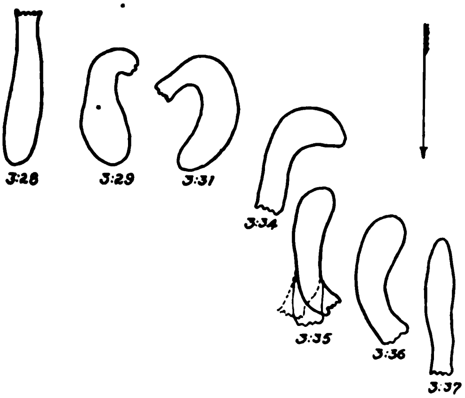


FIG. 2

Fig. 1 Stages in the orientation of H. captiva to moderately bright sunlight. Arrow shows direction of the light. The time at which each recorded observation was made is given.

Fig. 2 Conditions as in figure 1; a case which shows pseudo trial movements. The successive outlines have been shifted laterally for the sake of clearness.

The rapidity of orientation varies with the light intensity employed, a higher intensity giving a more rapid orienting effect (see Table 1).

Direct sunlight, or electric light of more than 200 c.m. intensity, has an almost immediate toxic action (Table 2). Animals exposed to light of these intensities were within 15 to 30 minutes caused to eviscerate through the anus or through dehiscent openings in the body wall.[6] Previous to the production of this moribund condition the animals attempted to orient, but before this reaction was completed they moved aimlessly, and later withdrew the tentacles, pedicels and papillae. After a quarter of an hour in this state very few recovered when removed to more favorable conditions.

TABLE 1

Time required for the completion of orientation by horizontal light parallel to the long axis of the animal acting on the anterior end, for individuals of different sizes; (a) with diffuse daylight, (b) with moderately bright direct sunlight. Tests were made at least an hour apart.

ANIMAL		ORIENTATION TIME, MIN.	
No.	Length, mm.	a	b
1	6	51	30
2	40	6	3
3	60	7	3
4	70	10	4
5	85	11	6

TABLE 2

Number of successive reactions to shadows, cast at 0.5 minute intervals, obtained from the anterior end of Holothuria captiva of different sizes before exhaustion; (a) in diffuse sunlight (b) after being in bright sunlight for 10 minutes.

ANIMAL		NO. OF REACTIONS	
No.	Length, mm.	a	b
1	25	3	0
2	40	7	0
3	43	3	0
4	44	4	0
5	45	4	0
6	53	18	2
7	56	8	1
8	58	16	1
9	62	9	1
10	68	19	4
11	75	19	3

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[6] Under these conditions evisceration through the anus took place without the discharge of the Cuvierian organs. Taken in connection with the results of certain of my experiments, this leads me to doubt the entire correctness of Mines's explanation of the discharge of these organs as due to internal pressure. A variation of the experiment he performed on H. nigra (Mines, 1912) consisted in artificially raising the internal pressure of H. captiva by pressing on it. Cuvierian organs may thus be caused to come out through the anus at will, but their appearing and the mode and extent of their subsequent elongation are then decidedly not normal.

IV. The only conclusion permissible from the above experiments is that the behavior of Holothuria captiva toward horizontal light is a clear and definite example of negative phototropism, in the sense intended by Loeb. The animal is sensitive to light; it is one of the few echinoderms thus far investigated which possesses a strong physiological polarity as regards movement and a functionally bilateral structure; it is compelled so to adjust itself in a field of light that the effect on its opposite sides is equalized.

What is the significance of these findings in interpreting the photic reactions of starfishes, ophiuroids and sea urchins which, in contrast to H. captiva, move toward, or away from, the light without orientation? In the forms which have hitherto been studied, there is no pronounced structural or physiological prominence of an anterior end (cf. Cole 1913). They may, then, be regarded as animals which are phototropic, but in which the part which is the effective anterior changes with the altering of external (and internal) conditions. This explanation also

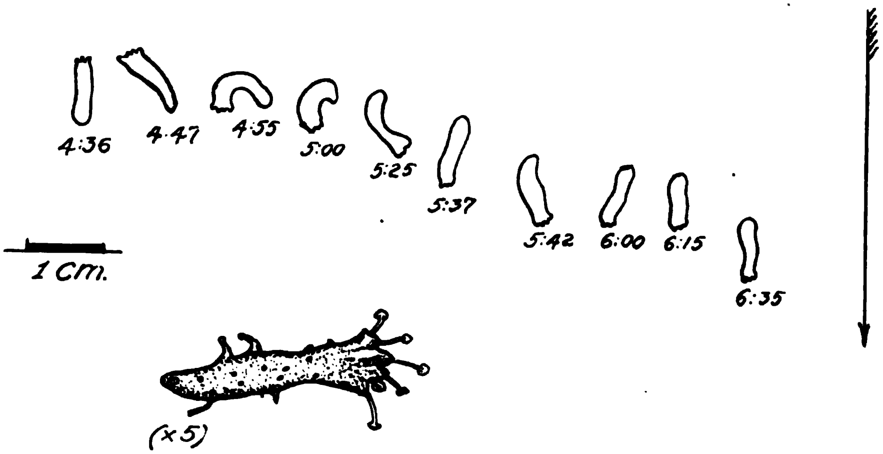

Fig. 3 Details in the orientation of an animal 6 mm. long, of which an enlarged dorsal view is given. Outlines separated laterally to avoid confusion of lines.

applies to holothurians like Thyone (Pearse 1908)[7] and (in my experiments) Cucumaria punctata. These animals are not nearly so sensitive as H. captiva, nor is their structural bilaterality so marked; in particular, functional pedicels are in Thyone found all over the body, and in Cucumaria on all five radia.

V. Holothuria captiva is likewise sensitive to shading (Table 2). It reacts negatively over its whole surface to sudden decrease in light intensity, but is not reactive to an increase of intensity. The tentacles are the most sensitive; the order of sensitivity of the parts of the animal is the same as that for the direct action of light. It is probable, therefore, that a single photoreceptive system is present, which is capable of responding to both kinds of stimulating agencies.

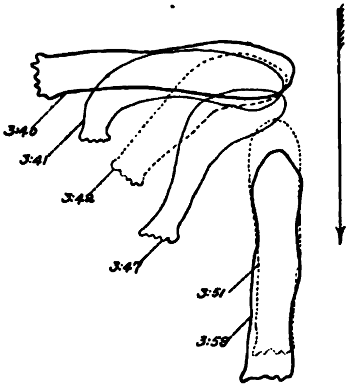

Fig. 4 Orientation by lateral light.

A detailed review of the literature on "differential sensitivity"[8] would consist almost entirely in a mere catalog of the reactions of a variety of animals to shading, or to increase in light intensity, or, rarely, to both. Such reviews may be found in the books of Mast (1914), Kafka (1914), and similar works. With the exception of v. Uexküll's papers (1897, 1900), there are very few recorded facts of physiological significance, so far as an understanding of the mechanism involved in stimulation is concerned. It may be pointed out, however, that writers on this subject—Rawitz (1888), Nagel (1896), and particularly Hargitt (1909) and Verworn (1913)—have been troubled by the fact that a shadow, "the negation of light," can produce a positive sensory effect. The difficulty, as is clear from the discussion of Hargitt (1909), and Verworn (1913, pp. 41 et seq.), results from a too superficial view of the nature of a "stimulus." This may be seen in the following quotation from Verworn (op. cit., p. 44):

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[7] Scott (1914) speaks of Thyone as being "oriented by light," but seems to use the word in a very loose sense.

[8] "Differential sensitivity" is personally preferred to Bancroft's "differential sensibility" (Bancroft, 1913); both are equivalents of "Unterschiedsempfindlichkeit."

> . . . . It is altogether impracticable to define the stimulus itself in relation to the nature of the effect which the stimulus has upon the substances in the system. One can only appreciate the nature of stimulation in relation to the vital conditions and without considering the nature of the action of the stimuli on the living substance.

So far as I can discover, this statement is physiologically meaningless. It is not out of place, in this connection, to insist upon the simplicity of the view that when a sensory cell is aroused to activity the real *stimulus* is the changed physico-chemical condition within the cell, or at its surface membrane.

The integument of Holothuria is provided with pigment cells, in relation with which there terminate fibers of the radial nerve strands. Polara (1906) suggested that these were the organs of a diffuse photic sense. If we picture, possibly located in these cells, a balanced system including (1) a photosensitive material, (2) the precursors which in the normal course of metabolism go to produce it, and (3) the products of its photolytic decomposition, which may or may not be identical with its precursors, we have all the essentials of a mechanism with which to account for the photic reactions of Holothuria. The photolysis of the substance provides the stimulus for photokinetic reactions and phototropism, while the abrupt cessation of this photolytic action, or the re-institution of reconstructive reactions previously inhibited by light, is the stimulus for the shading reflex. This view is consistent with the electrical behavior of the excised vertebrate eye under similar conditions (Einthoven and Jolly, 1908).

I was at first of the opinion that possibly the green skin pigment was itself the photosensitive material. It is fluorescent, with a bluish-green light like uranium glass, and water or alcohol extracts containing it are bleached by exposure to sunlight and air. But this conception must be modified in view of subsequent experiments. Fresh neutral or alkaline watery extracts of the skin of Holothuria contain this pigment and an abundant supply of a catalase-like enzyme. They are not bleached rapidly enough, even with the addition of hydrogen peroxide, to warrant the belief that the pigment is normally decomposed by light to any great extent. But such preparations show a notable acceleration in the evolution of oxygen from hydrogen peroxide when they are exposed to bright sunlight, which disappears on return to the shade. It is not too extreme to suggest that there is some relation between this increase in peroxide catalysis and the fact that nerve processes terminate in connection with the surface of the pigment cells. R. S. Lillie (1913) has shown that in frog leucocytes the most rapid oxidations occur at the nuclear and plasma membranes, and that these oxidations are increased by stimulation.

The fluorescent pigment therefore probably acts merely as a sensitizer. The theory of the intimate connection of this pigment with photoreception is supported, not only by the widespread occurrence of fluorescent substances in animal photosensitive organs, and the well-known influence of such materials in increasing the toxic action of light when they are injected, but also by the following observations which I have made upon cases occurring in nature:

(*a*) H. surinamensis is a nocturnal animal. In its normal habitat it comes during the night to the surface of the sandy mud in which it lives. When found, as is occasionally the case, among rocks, the surface of the body, excepting the tentacles and podia, is covered by a thin firm layer of dark silt held by a mucoid secretion. In the summer of 1914 I secured four individuals of this species which were found on the upper surface of stones in fairly bright sunlight. They were 6 to 10 cm. in length. These specimens were devoid of greenish coloring matter, the only pigment visible being the dark brown melanoid which usually accompanies the fluorescent material. Though kept in the laboratory for some days, these animals were found to be totally insensitive to shading and only vaguely photokinetic. They did not orient to light.

(*b*) H. surinamensis and small H. captiva are sometimes found having unmistakably regenerated anterior or posterior ends.[9] Such portions are notably deficient in pigmentation and at the same time give evidence of a lower sensitivity to light and shading than that possessed by the normally pigmented tissues.

(*c*) In the three Bermudan species of Holothuria the order of increasing sensitivity to light and shading—H. rathbuni < H. surinamensis < H. captiva—is exactly that of the relative increasing amount of fluorescent green pigment in their skins.

(*d*) It is a general rule, certainly true of holothurians in other responses, that the activity of an animal is inversely proportional to its size. Comparing the number of shading reactions obtained from animals of different sizes (Table 2), it will be seen that the smaller individuals, which react, in general, more vigorously, are more quickly exhausted. The pigmentation of the very young H. captiva (about 4–6 mm. long), however, is less dense than that of older specimens, and these are much less sensitive to photic stimulation, both in the shading reflex and in photokinesis. The relative times occupied in orientation to light of standard intensity will illustrate this point (see the trails in figures 1 and 3, and Table 1).

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[9] These structures in H. surinamensis can be duplicated by regeneration after experimental cutting. In nature they are probably the result of autotomous bisection. I can confirm for H. captiva the statement of Dalyell (1851) that young holothurians undergo spontaneous self division. Larger specimens of the species last mentioned do not show naturally occurring regeneration and do not regenerate in the laboratory. In this respect H. captiva resembles the majority of the genus (Torelle, 1909).

SUMMARY

1. Holothuria captiva Ludwig is photokinetic. Its whole surface is sensitive to light and to shading. It gives no reaction to an increase in light intensity. The order of sensitivity of its parts is: anterior end > posterior end > podia > mid-body surface.
2. Light above 200 c.m. intensity exerts a distinctly toxic influence.
3. Like H. surinamensis, the animal moves only with the anterior end in advance.
4. Unlike starfishes, sea-urchins, and less pronouncedly bilateral holothurians, it is oriented by light. It is negatively phototropic.
5. The photoreceptive mechanism includes the action of the green fluorescent integumentary pigment as sensitiser.

REFERENCES

Bancroft, F. W. 1913 Heliotropism, differential sensibility, and galvanotropism in Euglena. Jour. Exp. Zoöl., vol. 15, p. 383–428, 5 figs.

Cole, L. J. 1913 Direction of locomotion in the starfish (Asterias forbesi). Jour. Exp. Zoöl., vol. 14, p. 1–32, 9 figs.

Crozier, W. J. 1914(?) The sensory reactions of Holothuria surinamensis Ludwig. Zoöl. Jahrb., Abt. Physiol.

Dalyell, J. G. 1851 The Powers of the Creator Displayed in the Creation, etc., vol. 1, London, 286 p., 70 Pls.

Einthoven, W., and Jolly, W. A. 1908 The form and magnitude of the electrical response of the eye to stimulation by light at various intensities. Quart. Jour. Exp. Physiol., vol. I, p. 373–416, 25 figs.

Hargitt, C. W. 1909 Further observations on the behavior of tubicolous annelids. Jour. Exp. Zoöl., vol. 7, p. 155–188.

Kafka, G. 1914 Einführung in die Tierpsychologie: Bd. I, Die Sinne der Wirbellosen; vii + 593 p., 362 figs., Leipzig.

Lillie, R. S. 1913 The formation of indophenol at the nuclear and plasma membranes of frog's blood corpuscles and its acceleration by induction shocks. Jour. Biol. Chem., vol. 15, p. 237–248, 1 Pl.

Mast, S. O. 1911 Light and the Behavior of Organisms. xi + 410 p., 36 figs., New York.

Mines, G. R. 1912 Note on the mechanism of discharge of the Cuvierian organs of Holothuria nigra. Quart. Jour. Micr. Sci., vol. 57, p. 301–304, 1 Pl.

Nagle, W. A. 1896 Der Lichtsinn augenloser Thiere; 120 p., Jena.

Pearse, A. S. 1908 Observations on the behavior of the holothurian, Thyone briareus (Les[u]eur). Biol. Bull., vol. 15, p. 259–288, 6 figs.

POLARA, G. 1906 Sulla connessione delle cellule pigmentate del mesenchima cutaneo dell olothurie con i nervi cutanei e sul lore significate funzionale. Boll. Accad. Gioenia, Catania, Fasc. 88, p. 12–24, 2 figs.

RAWITZ, B. 1898 Der Mantlerand der Acephalen. Jena. Zeit. f. Naturwiss. Bd. 22, p. 415–556, 1 fig., 6 Taf.

SCOTT, J. W. 1914 Regeneration, variation and correlation in Thyone. Amer. Nat., vol. 48, p. 280–307, 5 figs.

TORELLE, E. 1909 Regeneration in Holothuria. Zool. Anz., Bd. 35, p. 15–22.

UEXKÜLL, J. v. 1897 Der Schatten als Reiz für Centrostephanus longispinus Zeit. f. Biol., Bd. 34, p. 319–339.

1900 Die Wirkung von Licht und Schatten auf die Seeigeln. Zeit. f. Biol., Bd. 40, p. 447–476.

VERWORN, M. 1913 Irritability; xii + 264 p., 62 figs. New Haven.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

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No. 35.

ON THE NUMBER OF RAYS IN ASTERIAS TENUISPINA LAMK. AT BERMUDA.

By W. J. Crozier.

From The American Naturalist, Vol. XLIX, No. 577.

CAMBRIDGE, MASS., U. S. A.
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[Reprinted from The American Naturalist, Vol. XLIX., Jan., 1915.]

CONTRIBUTIONS FROM THE ZOOLOGICAL LABORATORY
OF THE MUSEUM OF COMPARATIVE ZOOLOGY AT
HARVARD COLLEGE, NO. 256.

ON THE NUMBER OF RAYS IN ASTERIAS TENUISPINA LAMK. AT BERMUDA[1]

BY W. J. CROZIER

Harvard University

I. It was suggested by Clark (1901) that the average number of rays borne by *Asterias tenuispina* was different for separate localities in Bermuda. He examined forty specimens of this species—eleven from Coney Island and twenty-nine from Harrington Sound; in the first set the average number of rays was 5.91 (I find the mode to be 6), in the second set 6.93 (with a mode of 7). If this condition really obtains, it would be an exceedingly interesting matter to determine the factors responsible for this sort of difference. I have therefore examined a number of *Asterias* (312 in all) from several localities in the Bermudas, namely: Agar's Island, Spanish Point, Hawkins Island, Ely's Harbor, Hungry Bay, Harrington Sound and Coney Island; the first four are situated on the periphery of Great Sound, the others at widely removed points on the north and south shores. For the identification of these places, references may be made to the maps published by Mark (1905).

These observations were made at the Bermuda Biological Station, mostly during the summer of 1914.

II. The first lot of starfishes was collected in the immediate vicinity of Agar's Island in 1913. The number of rays varied from 2 to 9; the frequency distribution is given in Fig. 1. The modal number of rays is clearly 7. In 1914 a collection of *Asterias* from this place gave the ray frequency distribution shown in Fig. 2, where the modal number of rays is again 7. Collections, during 1914, at the other stations named gave the following ray frequency counts:

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[1] Contributions from the Bermuda Biological Station for Research, No. 35.

Station and Year	No. of Specimens	Modal Ray Number	See Figure
Agar's Isl., 1913	43	7	1
Agar's Isl., 1914	62	7	2
Spanish Point, 1914	33	7	3
Hawkins Isl., 1914	39	7	4
Ely's Harbor, 1914	36	7	5
Hungry Bay, 1914	41	7	6
Coney Island, 1914	20	7	7
Harrington Sound, 1914	38	7	8

The modal number of rays is in each case 7. This is true for the same locality in two successive years, for near-by localities and for places widely enough separated to yield critical data relative to the suggestion which prompted this inquiry. For the total population examined the ray frequency distribution, which of course gives a mode of 7 rays, is plotted in Fig. 3. It is to be noted further that according to Ludwig (1897, p. 345) the most common number of rays in *A. tenuispina* from the Mediterranean is also 7.

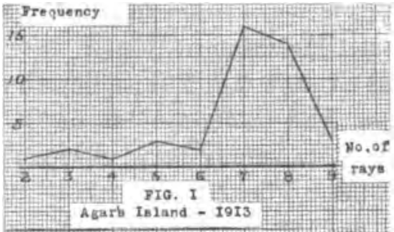


FIG. 1
Agar's Island - 1913

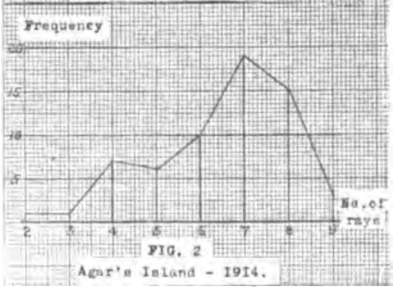


FIG. 2
Agar's Island - 1914.

III. It has been observed by every one who has studied *A. tenuispina* that in most of the individuals the rays occur in two groups, those of one group being longer than those in the other, though within each group the rays are of about the same length. This condition is evident in 259 (83.6 per cent.) of my specimens. There is general agreement (cf. Ludwig, 1897, and Ritter and Crocker, 1900), that in some cases, if not in all, "this disparity in size is due to the regeneration of halves of automatically bisected animals." My observations fully confirm this. I have witnessed, as did Ludwig, several cases of spontaneous self-division in the laboratory. The casting off of one or more rays may at any time be induced by holding or injuring one or several rays, or by the stimulation of a ray with dilute acid applied with a pipette. The autotomy of a single ray takes place very much as described by King (1898) for *Asterias vulgaris;* the existence of a "breaking joint" in the region of the fifth ambulacral

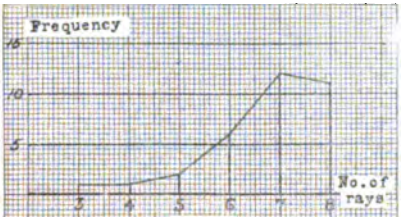


FIG. 3

Spanish Point

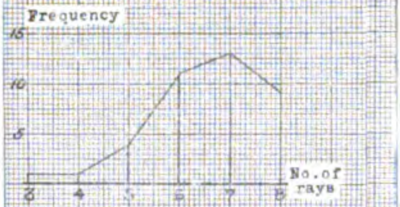


FIG. 4

Hawkins Island

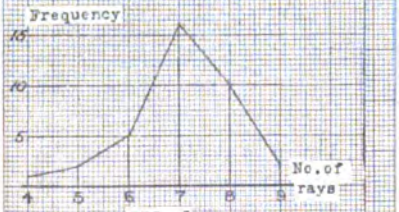


FIG. 5

Ely's Harber

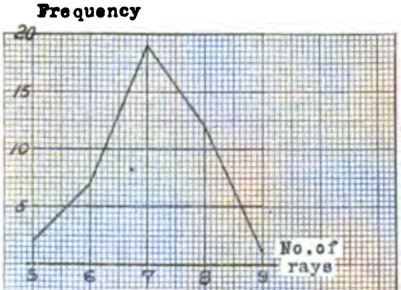


FIG. 6

Hungry Bay

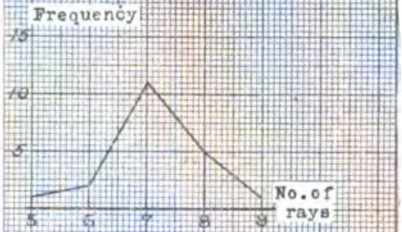


FIG. 7

Coney Island

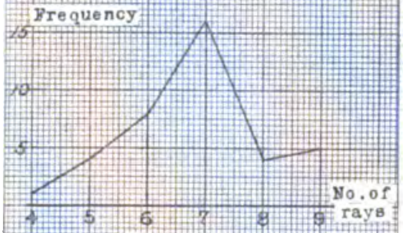


FIG. 8

Harrington Sound

ossicle is shown by the fact that even in preserved material the rays part very easily in that region.

The relative abundance of cases in which there are evident two groups of rays of different length indicates that, as in *Linckia* (Clark, 1913), autotomous division is a normal method of asexual reproduction.

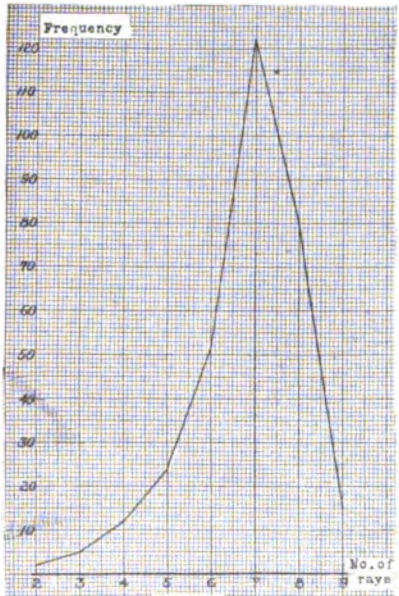


FIG. 9

Distribution of ray frequencies in the total population.

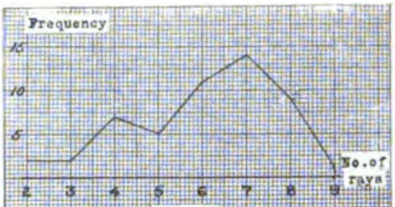
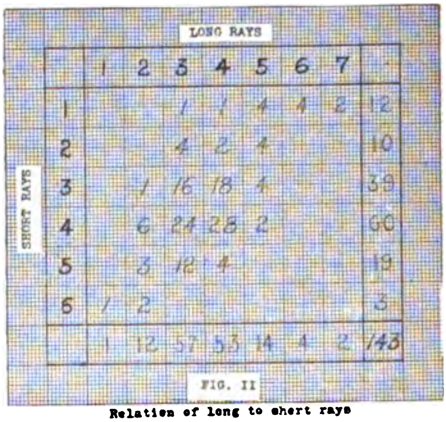


FIG. 10

Distribution of ray frequencies among individuals with all rays of equal or nearly equal length



LONG RAYS

SHORT RAYS	1	2	3	4	5	6	7	
1			1	1	4	4	2	12
2			4	2	4			10
3		1	16	18	4			39
4		6	24	28	2			60
5		3	12	4				19
6	1	2						3
	1	12	57	53	14	4	2	143

FIG. 11

Relation of long to short rays

IV. The numerical relations of the old rays to the regenerating ones, and the topographical arrangement of the latter, yield evidence relative to certain questions in the physiology of regeneration.

(*a*) It is to be observed that the regeneration in question has taken place apart from experimental control; therefore information as to the number of rays usually present just before *Asterias* undergoes self-division must be deduced from the data at hand. The modal-ray frequency for specimens with rays of very nearly equal length is 7 (Fig. 10), but it is a question whether this appearance of equality in ray length may not be due to a variety of conditions, especially the rapid growth of regenerated rays. The regenerating rays of *Linckia* (Clark, 1913; Monks, 1904) and the newly formed rays of multiradiate types (Ritter and Crocker, 1900) grow more rapidly than the old ones and soon reach the dimensions of the latter; this is also indicated in my series. But the correlation of the number of long with the number of short rays (using only those cases in which the two groups were clearly distinct) makes it evident (Fig. 11) that the condition in which there are 3 or 4 long rays and 4 short ones is by far the most common; and further, that the cases in which there are either 3 or 4 long rays are almost equally abundant. It seems not unlikely, then, that *A. tenuispina* usually has 7 rays before it divides, and that it divides into two parts having, respectively, 3 and 4 rays, the division-surface then giving rise, in the greater number of cases, to 4 new rays, but sometimes to 5, 3 or even 2.

If all the individuals observed had undergone autotomy and regeneration according to this scheme, then those with 7 and those with 8 rays would be expected to occur in equal abundance; 8 is next in frequency to 7, but the latter preponderates because some starfishes have probably not autotomized at all, and because all the animals which have divided do not adhere to this paradigm (see Fig. 11). Yet, in the majority of cases, 4 rays are regenerated whether there are 2, 3 or 4 long (old) rays in evidence.

It would seem that self-division may occur at any time in the life history of *A. tenuispina,* or at least in animals of all sizes, though it is my impression, gained from handling many live individuals, that the smaller (younger ?) ones autotomize more readily than larger ones. Those showing two distinct ray groups ranged in longer ray length from 11 mm. to 65 mm. There is no evidence that autotomous divisions follow one another rapidly, or indeed that they occur more than once in any given individual.

One case was observed in which there was one long ray only, and 6 shorter ones. This may mean that a single ray can regenerate the whole body, as suggested by v.

Martens (1866, quoted by King, 1898) for this species. I have not been able to substantiate this idea by laboratory experiments, for, in my tests, single isolated rays did not live more than a few weeks.

(*b*) Newly forming rays have a tendency to appear in symmetrically disposed pairs (see Fig. 12), which gives to many individuals a strikingly bilateral aspect. This is accentuated by their behavior, for, in the absence of directive stimuli, they commonly move with the longest rays in advance. In moving away from the light, the locomotor movement of the group of longer rays also tends, in many cases, to produce a spurious "orientation." When placed oral side up, the larger rays exert a determining influence on the direction and manner of righting. These effects are due to the greater pedicel and muscle development of the longer, thicker, rays.

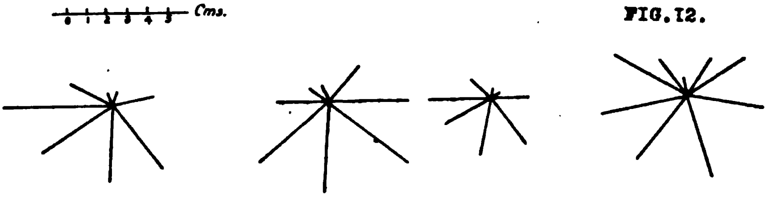


FIG. 12.

DIAGRAMATIC REPRESENTATION OF THE RAYS OF FOUR ASTERIAS, SHOWING TENDENCY OF RAYS TO APPEAR IN PAIRS. Measured from the mouth along the ventral side.

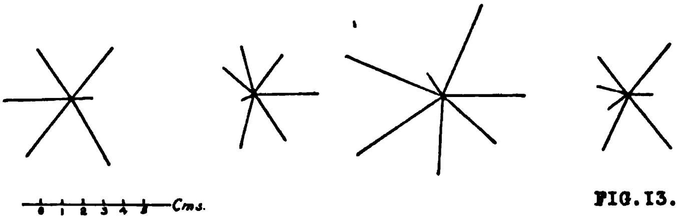


FIG. 13.

ILLUSTRATING THE RELATIONS OF NEW RAYS.

The formation of two rays at a radial cut on the disc was found by King (1900) in *Asterias vulgaris.*

V. I have suggested, above, that *Asterias* with 7 subequal rays have probably arrived at that condition by different routes. One method of ray multiplication appears to be the spontaneous addition of new rays at any point on the disc. Twelve starfish were found which showed but one ray markedly shorter than the others.

Of these, 4 had 5 long rays, 4 had 6, 2 had 7, 1 had 3 and 1 had 4 (see Fig. 11). The addition of new rays during adult life is, so far as known, unusual among starfish, excepting in the multiradiate forms (cf. Ritter and Crocker, 1900; Clark, 1907; M'Intosh, 1907). The twelve cases found in *A. tenuispina* may mean merely that a single ray has been cast off and is being regenerated, for there is found about the same percentage of naturally occurring regenerating examples of *A. vulgaris* (King, 1898; 1900). Yet I am inclined to interpret this condition as indicating the way in which the modal hepta-radiate form is derived from the fundamental penta-radiate one, or from a hexaradiate plan, if the young of *A. tenuispina* be like the postlarvæ of *Pycnopodia* (Ritter and Crocker, 1900) previous to self-division.[3] The three smallest *Asterias* seen had 6 rays. These were subequal and 8 ± mm. long. Other specimens, slightly larger, had either 7 or 8 rays.

Cases such as those illustrated in Fig. 13 may further prove that addition of new rays occurs independently of the reformation of rays subsequent to self-division.

VI. The number of madreporites in *A. tenuispina* is also variable, as noted by Ludwig (1897, p. 358) and others. The number of madreporic bodies is certainly not correlated with the size of the starfish. One of the smallest ones seen had 8 rays and 5 madreporites, its mean ray length being 10 mm.; while the largest animal collected had 5 rays, with a mean ray length of 70 mm., and but one madreporite. The table in Fig. 14, which includes all cases in which the madreporites were counted, shows that, while the distribution of these bodies is irregular, their number is to some extent correlated with the number of rays. Ludwig gave it as his opinion that there was no correlation of this sort. The relation stands out more clearly if only those individuals having equal rays (and therefore presumably "full grown") are included (Fig. 15). Unfortunately, the number of animals is small.

Multiple madreporites were noted in 5 out of 101 examples. Three of these showed a condition which might have arisen either by the fusion of two plates or by the division of a single one. The other two cases were similar, but of trefoil form. Dissection showed, in each instance, that a single stone canal was present. Therefore these multiple plates had probably arisen by the division of an originally single one. (For a similar condition in *A. vulgaris,* see Davenport [1901].) Only one multiple madreporite was found in any one individual.

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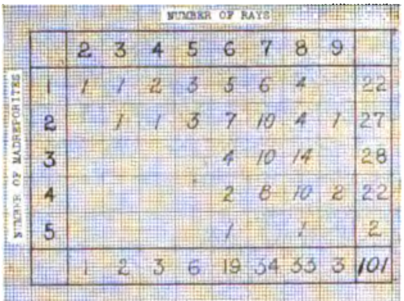
[3] According to Clark's (1907) studies, the young *Heliaster* has five rays only; his results throw considerable doubt upon the correctness of the conclusions of Ritter and Crocker.

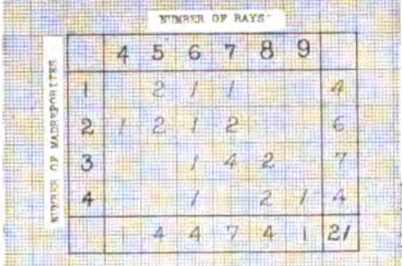


NUMBER OF RAYS

NUMBER OF MADREPORITES	2	3	4	5	6	7	8	9	
1	1	1	2	3	5	6	4		22
2		1	1	3	7	10	4	1	27
3					4	10	14		28
4					2	8	10	2	22
5					1		1		2
	1	2	3	6	19	34	33	3	101

FIG. 14

Correlation between number of madreporites and number of rays



NUMBER OF RAYS

NUMBER OF MADREPORITES	4	5	6	7	8	9	
1		2	1	1			4
2	1	2	1	2			6
3			1	4	2		7
4			1		2	1	4
		4	4	7	4	1	21

FIG. 15

Relation of ray frequency to number of madreporites in animals with rays of equal length

SUMMARY

1. The modal number of rays in *Asterias tenuispina* is 7. The range in ray number is from 2 to 9.

2. The 7-ray condition is uniformly the most frequent, even in widely separated localities.

3. The modal ray number is the same for animals with subequal rays as for those with a group of regenerating rays.

4. The evidence indicates that, *most commonly, A.*

tenuispina has 7 rays before it undergoes autotomy, that it divides into 3-ray and 4-ray portions, and that each of these parts regenerates 4 rays.

5. Regenerating rays tend to appear in bilaterally disposed pairs, as regards size.

6. There is no evidence that self-division occurs often in the life of individuals, though possibly it does.

7. New rays may be added at any point on the disc.

8. The number of madreporites varies from 1 to 5, and is to some extent correlated with the number of rays; it is not correlated with the size of the animal.

9. Double or triple madreporites occur in about 5 per cent. of the individuals.

REFERENCES

Clark, H. L. 1901. Bermudan Echinoderms. A Report on Observations and Collections Made in 1899. *Proc. Boston Soc. Nat. Hist.*, Vol. 29, No. 16, pp. 339–345.

1907. The Starfishes of the Genus *Heliaster*. *Bull. Mus. Comp. Zoöl.*, Vol. 51, pp. 23–76, 8 pls.

1913. Autotomy in *Linckia*. *Zool. Anz.*, Bd. 42, No. 4, pp. 156–159.

Davenport, G. C. 1901. Variation in the Madreporic Body and Stone Canal of *Asterias vulgaris*. *Science*, N. S., Vol. 13, pp. 374–375.

King, Helen D. 1898. Regeneration in *Asterias vulgaris*. *Arch. f. Entw.-mech.*, Bd. 7, Heft 2–3, pp. 351–363.

1900. Further Studies on Regeneration in *Asterias vulgaris*. *Arch. f. Entw.-mech.*, Bd. 9, Heft 4, pp. 724–737.

Ludwig, H. 1897. Die Seesterne des Mittelmeeres. *Fauna u. Flora d. Golfes v. Neapel*, Monogr. 24 x + 491 p., 12 Fig. and 12 Taf.

M'Intosh, D. C. 1907. Meristic Variation in the Common Sun-Star (*Solaster papposus*). *Proc. Roy. Phys. Soc. Edinburgh*, Vol. 17, pp. 75–78.

Mark, E. L. 1905. The Bermuda Islands and the Bermuda Biological Station for Research. *Proc. Amer. Assoc. Adv. Sci.*, Vol. 54, pp. 471–501, 16 pls.

Martens, E.von. 1866. Ueber ostasiatische Echinodermen. *Arch. f. Naturg.*, Jahrg. 32, Bd. 1, pp. 57–88.

Monks, Sarah P. 1904. Variability and Autotomy of *Phataria*[1] [*Linckia*]. *Proc. Phila. Acad. Nat. Sci.*, Vol. 56, pp. 596–600.

Ritter, W. E., and Crocker, G. R. 1900. Multiplication of Rays and Bilateral Symmetry in the 20-rayed Star-fish, *Pycnopodia helianthoides* (Stimpson). (Papers from the Harriman Alaska Expedition, III.) *Proc. Wash. Acad. Sci.*, Vol. 2, pp. 247–274, pl. 13, 14.

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[1] "Phataria" is an error, as pointed out by Clark (1913).

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., 31(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., 56(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., 38(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., 31(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., 40(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., 54: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1906.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., 46(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., 41(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., 42(18): 461–486. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., 42(19): 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., 33(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., 43(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, 13 (2): 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., 44(5): 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., 44(23): 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., 7(2): 189–238. 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, 1910 (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., 36 (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., 46(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., 47(7): 217–226, 4 pl. July, 1911.
22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. 10: 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, 29(1): 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., 47(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773. 4 pl. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569–626. 3 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotiop of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. Crozier, M. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

VOL. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
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3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
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9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pl. Feb., 1907.

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13. **Cole**, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
14. **Morgulis**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
15. **Morgulis**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
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    Separate : 6 pp. Nov., 1909.
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19. **Smallwood**, W. M., **and Rogers**, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
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27. **Chester, W. M.** — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20): 737–773. 4 pl. May, 1913.
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33. **Crozier, W. J.** — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **Crozier, W. J.** — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1): 8–20. Dec., 1914.
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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 259.

THE ORIENTATION OF AMPHIOXUS DURING LOCOMOTION[1]

LESLIE B. AREY

Observers have differed regarding the question as to which end of Amphioxus is in advance during swimming. Rice ('80, p. 8) seems to have been the first to record observations on this subject:

> These movements were executed sometimes upon the back, sometimes upon the abdomen in the position of ordinary fishes, it seemed to make very little difference which side was uppermost, but I have never seen them move backwards or tail-end foremost. After circumnavigating the vessel once or twice gradually moving slower and slower, they would stop and sink down upon the sand at the bottom.

In a more general statement Steiner ('86, p. 497) came to the same conclusion as Rice, "sie stellen sich so auf, dass ihre Breitseite in die verticale Ebene fällt und rasch entfliehen sie (from the stimulus) mit grosser Geschwindigkeit, das Kopfende voran, indem der Körper schlängelnde Bewegungen macht, an denen der Kopf nachweisbar theilnimmt." Two years later ('88, p. 41) he expressed the same opinion in almost identical language.

Parker ('08, p. 441) took the opposite view:

> The locomotion of amphioxus is a rapid, curiously irregular wriggle, often accompanied with somersault-like movements which make it impossible to be sure at any moment whether the animal is swimming backward or forward. The results of momentary stimulation, however, show very conclusively that amphioxus can swim both backward and forward, and that the direction of swimming at the beginning of any course is dependent upon the part of the animal's body that was stimulated. But how long amphioxus keeps to one form of movement I was unable to discover. The fact that it usually buries itself in the sand tail first leads me to believe that, though it can swim forward, as maintained by Rice and by Steiner, it usually swims backward.

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[1] Contributions from the Bermuda Biological Station for Research. No. 36.

Parker and Haswell ('10) in their "Textbook of Zoology" refer to the more or less upright position which Amphioxus assumes after burrowing in sand and then add the following astonishing and somewhat ambiguous statement (vol. 2, p. 46): "It also swims in the vertical position, - - - - - - ." However this assertion may be interpreted, it certainly is contrary to fact as far as the West Indian Amphioxus is concerned, and from the description of other writers (see also the frontispiece in Willey's '94 book), the same criticism undoubtedly applies to the closely allied European species as well. Anyone may easily convince himself that the foregoing quotation either presents a gross error or is highly misleading (according to the alternate possibilities of interpretation), if he will observe for a short time the locomotor responses, regardless of the antero-posterior orientation, exhibited by Amphioxus under various natural or experimental conditions.

While working recently at the Bermuda Biological Station opportunity was afforded me for making observations on the swimming habits of the West Indian lancelet, Branchiostoma caribbaeum Sundevall, a species very similar to the common European Amphioxus. This animal is found in abundance in the coarse coral and shell sand of Flatts Inlet, which connects the water of Harrington Sound with the outside ocean.

Ordinary mechanical stimulation, as by a finely drawn glass rod, gave too active a response for observation and accordingly a milder stimulus was sought. This was found in a weak stream of sea-water forced from a rather large canula; if the jet was weak and was directed vertically through 10 cm. of air and 10 cm. of water, the entire body of the animal became subjected to a gentle stimulus formed by the wave front. When thus stimulated the locomotor response tends to be less energetic and the disadvantages of local or directional stimulation are obviated, while it has a further advantage over other mild stimuli such as jarring the containing-dish, inasmuch as individual animals may be singled out for experiment and watched from the beginning of their course.

About thirty individuals were placed in three glass jars containing a layer of 'amphioxus sand' and were stimulated by the method just described. All responses in which the orientation was doubtful and those responses which included wild dashes or excessive somersaulting were disregarded; of the responses recorded, some were observed during the whole course and others were judged chiefly by the orientation at the beginning and particularly at the slowed-down finish of the swimming reaction. A tabulation of observations obtained in this manner is as follows:

Total number of responses	50
Anterior end in advance	41
Posterior end in advance	9

In a considerable number of animals the first movement was backward, but the direction was quickly reversed bringing the anterior end in advance; this condition will be discussed later in connection with other experiments.

When, however, Amphioxus are free to move in unlimited space, the somersaulting and the quick reversals of direction make accurate observations as to what is occurring during the response very difficult. To obviate this difficulty a large porcelain pan was partly filled with water, giving depths which varied from 0.5 cm. at the edge to 1.25 cm. at the center. The movements of Amphioxus under these conditions were as follows: When a stimulus (a bristle or finely drawn glass rod) was applied to the anterior end, the animal responded with a backward spring; if locomotion was now continued, this direction was not maintained for more than a few centimeters, for a quick reversal occurred, exceedingly difficult to follow, but which seemed to include a doubling end for end succeeded by a partial rotation of the long axis of the animal about its middle point; as a result the Amphioxus swam away with its anterior end in advance and usually at an acute angle with the direction toward which it was heading before stimulation occurred. During subsequent swimming these reversals occasionally occurred and as a result the posterior end of the animal might be in advance for a short time; in this event, however, another reversal soon restored the former orientation and the normal direction of continued movement was plainly with the anterior end in front. When the posterior end was stimulated the animal sprang forward and if it continued to swim, it proceeded head foremost, although subsequent reversals usually occurred from time to time.

One lancelet of this set was especially instructive; after stimulation and the usual energetic response, it would continue swimming at a rate of about 1 cm. per second for a considerable distance without reversal; in several instances it more than circumnavigated the dish, a distance of over a meter, yet the anterior end was always carried in advance. Several other animals showed the same behavior but in a less degree; in general, after several responses the reaction was as long but less vigorous than that of a rested animal, and hence was easier to observe.

A circular trough 30 cm. in diameter, 0.5 cm. wide at the bottom and 1.25 cm. wide at the top was constructed by placing a porcelain pan, bottom up, inside a slightly larger pan; the purpose of this arrangement was to give the animal free swimming room but to limit its locomotion to one direction. The results were in agreement with those described previously; it was impossible to make an Amphioxus swim backward for more than a few centimeters before somersaulting and forward locomotion occurred.

A final method, which gave more conclusive evidence concerning the orientation of the animal during normal locomotion in unlimited space, consisted in treating one end of the lancelet's body with an *intra vitam* stain, whereby through direct observation one could be certain of the animal's orientation even during the wild dashes that often occur. Objections may be raised to the artificial conditions of the experiments described above, which were devised for limiting the animal's movements; thus it is entirely possible (although I do not believe it to be actually the case) that Amphioxus has a more complicated swimming behavior in the open than when locomoting in close quarters where some of its movements, including perhaps backward swimming, are omitted. The whole locomotor response is undoubtedly much more rapid during unimpeded progress, hence it might be argued that although the animal in slower and more deliberate swimming carried its anterior end persistently in advance, yet when moving rapidly, if once reversed to backward swimming (and I have shown above that this reversal actually occurs from time to time), the physiological inertia of the animal as it travelled at its highest speed would tend to keep it so oriented until the swimming movements grew less energetic and the animal returned to the deliberate swimming that is characteristic at the end of the course. The data given at the beginning of the paper, in which nine out of fifty animals were recorded as oriented with the posterior end in advance while swimming freely in large aquarium jars, would tend to strengthen this suspicion; on the contrary, the actual results obtained from a study of stained animals did not substantiate such a line of reasoning.

If an animal, with the exception of one end, is wrapped in a fold of wet absorbent cotton and laid on a glass plate, the exposed end can be immersed without difficulty in the stain; in this case a weak solution of neutral red made up in sea-water was used. The anterior end was the one stained in most cases, for the more open structure of the pharyngeal region offers a larger surface for the reception of stain. After the stain had caused coloration to a deep pink or light reddish shade the animals were allowed to recover over night.

When stimulated after such treatment, the stained extremity could be followed with comparative ease, and observations made in this way corroborated my earlier conclusions. Amphioxus does not locomote backward for any considerable distance, even when the response is extremely vigorous; but, after a somersault brings it tail-end in advance, either another reversal follows directly or the animal changes its course and returns more or less in the direction from which it came.

I believe the observations recorded in the first experiment of nine lancelets, which were supposed to swim backward, can be explained as follows: When the swimming response is nearing completion, the vigor of the muscular movements rapidly decreases and ends in complete collapse (Rice '80, p. 8; Parker '08, p. 441). After cessation of movement the animal is carried on a short distance by its own momentum and then sinks slowly to the bottom. According to notes taken at the time, five of the nine animals thus observed were judged chiefly by the finish of their response; if a reversal occurred just previous to the cessation of swimming, it is reasonable to expect that the nearly exhausted animal would not reverse again but would continue tail first with the last feeble strokes which precede complete exhaustion.

When Amphioxus has been kept in the laboratory for a short time the anterior half of many animals begins to turn pink and in a few days that end may become decidedly colored. This is presumably a manifestation of an approaching moribund condition, although the reactions of the lancelets appear to be practically normal. Observations of a number of Amphioxus in this condition led to conclusions similar to those gained by the study of artificially stained animals.

Referring to the quotations above, it will be seen that the views of Rice and of Steiner, although agreeing with mine in the main, show some differences. Rice's statement that he never saw an Amphioxus move 'tail-end foremost' is not only contrary to the results given in my tabulation, where nine out of fifty observed animals were so oriented during normal swimming, but is also not in accordance with Parker's ('08, p. 431; pp. 437–440) experiments, in which resting animals stimulated mechanically or chemically on the anterior end or mid-body, responded with a backward spring. Steiner's simple statement that Amphioxus locomotes 'das Kopfende voran' is certainly too general and omits entirely any mention of the characteristic backward movements just referred to in the criticism against Rice. My own observations agree perfectly with Parker's, to the effect that Amphioxus burrows in the sand tail first, but his belief, obtained as an inference from this habit, that the animal usually swims backward is directly opposed to the conclusion reached by me.

It is interesting, however, to see how near Parker was to the real solution of the matter, although it must be said that the whole question was not of major importance in his work; thus ('08, p. 431) he says:

> When the anterior end of an amphioxus resting in a shallow dish of sea water was touched even lightly with the bristle, the animal usually sprang backward, though occasionally forward. The backward spring was often accompanied by a somersault-like movement, whereby the animal became turned end for end. When the stimulus was applied to the posterior part of the body, the result was almost invariably a forward leap.

The somersaulting is only mentioned by him in connection with the backward spring, and this I have shown is characteristically present at the time of reversal to normal swimming, while in a forward leap it is unnecessary and is usually omitted.

The animals mentioned above, which swam slowly for a considerable distance in a pan of shallow water, afforded an opportunity to observe the movements of the body during locomotion. The head and tail were bent simultaneously toward the same side; the posterior of all the flexures, which is by far the most prominent, occurs approximately at the level of the atriopore; the next prominent flexure is at about the region of the first gonadic pouches but is much less extensive than the former. When swimming slowly no other flexures are evident except a suggestion of one rather close behind the anterior flexure last described. The occurrence of the two largest flexures just anterior and posterior to the gonadic pouches suggests that these pouches materially increase the rigidity of the body throughout the region where they occur and thus actually determine the position of the major flexures. As might be expected, when a forward spring occurs the first flexure is initiated at the anterior end and muscular activity extends posteriorly like a wave; when an animal leaps backward the reverse is true.

As regards orientation during locomotion, I thus conclude that while Amphioxus can swim backward for short distances, its normal orientation in continued swimming is with the anterior end in advance.

LITERATURE CITED

RICE, H. J. 1880 Observations upon the habits, structure, and development of Amphioxus lanceolatus. Amer. Nat., vol. 14, no. 1, pp. 1–19, pls. 1–2; no. 2, pp. 73–95.

STEINER, J. 1886 Ueber das Centralnervensystem des Haifisches und des Amphioxus lanceolatus, und ueber die halbcirkelförmigen Canäle des Haifisches. Sitzungsber. kgl. Preuss. Akad. Wiss., Berlin, Jahrg. 1886, Halbbd. 1, pp. 495–499.

1888 Die Functionen des Centralnervensystems und ihre Phylogenese. Zweite Abtheilung: Die Fische. Braunschweig, 8 vo., XII + 127 pp.

PARKER, G. H. 1908 The sensory reactions of Amphioxus. Proc. Amer. Acad. Arts and Sci., vol. 43, no. 16. pp. 415–455.

PARKER, T. J., AND HASWELL, W. A. 1910 A text-book of zoology. Vol. 2, Macmillan, London, 8 vo., XX + 728 pp., 537 figs.

WILLEY, A. 1894 Amphioxus and the ancestry of the vertebrates. Macmillan, New York, 8 vo., XIV + 316 pp., 135 figs.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. **Gulick**, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July-Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
6. **Mark**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **Barbour**, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
8. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–343, 1 pl. Sept., 1905.
9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. **Linton**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
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13. **Cole**, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
14. **Morgulis**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908-
15. **Morgulis**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
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18. **Smallwood**, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1) : 137–145. June, 1910.
19. **Smallwood**, W. M., and **Rogers**, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
20. **Carpenter**, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6) : 147–162, 1 pl. Oct., 1910.
21. **Esterly**, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7) : 217–226, 4 pl. July, 1911.
22. **Cowdry**, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10** : 1–53, 4 pl. July, 1911.
23. **Gross**, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. **Smallwood**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18): 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23** (4): 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20): 737–778. 4 pl. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10): 569–626. 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1): 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6): 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3): 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1): 8–20. Dec., 1914.
35. Crozier, M. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577): 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1): 37–44. July, 1915.

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3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
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31. **Parker, G. H.** — The Locomotion of Chiton. 2 p. April, 1914.
32. **Parker, G. H.** — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. No. 258

A NOTE ON THE PHYSIOLOGY OF THE CUVIERIAN ORGANS OF HOLOTHURIA CAPTIVA LUDW.[1]

W. J. CROZIER

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CONTENTS

I. Introduction.
II. Stimuli which lead to Cuvierian organ expulsion.
III. Mechanism of discharge.
IV. The adhesive property.
V. Summary.

I. Concerning the function and mode of action of the Cuvierian organs of holothurians there is available very little experimental evidence.[2] The following observations upon the physiology of these structures in one of the Bermudan species of Holothuria may therefore be of interest.

Holothuria captiva does not bury itself in mud or sand, but is to be found (during the day-time at least) only on the under surface of slabs of stone, in places where numbers of such stones occur piled together just under low-water level.[3] It clings to the rock surfaces with such tenacity that frequently numbers of pedicels must be torn before the animal can be removed. During the process of collection there almost invariably occurs the expulsion of some of the Cuvierian organs, which in this species are very abundant (cf. Clark, 1901, p. 342). I have never seen a case in which the tubes were in an extruded condition when the rocks bearing H. captiva were turned over under water, though in all several hundred animals were observed in their natural position. I can therefore offer no suggestion as to their rôle in the normal economy of the animal.

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[1] Contributions from the Bermuda Biological Station for Research. No. 37.

[2] For an account of the views of varius writers see Minchin (1892) and Mines (1912).

[3] Such localities were. Hungry Bay, near the entrance; Bluck's Point; and the old break-water near Walsingham, Castle Harbor. Previous papers (Crozier, 1914 a [?] and 1914 b) contain data on the behavior of these animals. The present account is based upon the experiments during the summers of 1913 and 1914, at the Bermuda Biological Station.

II. It has generally been stated that holothurians possessing Cuvierian organs expel them when the animal is "irritated;" presumably mechanical stimulation of an intense character is meant. H. captiva was observed to discharge its tubes when the back or side was stimulated by repeated gentle touches with a blunt instrument, or when the papillae or tentacles were pinched. The brim surrounding the dorsally situated anus became at the same time elevated into a funnel-shaped tube, the opening of which was, in a general way, directed toward the irritated area. The number of organs discharged varied with the intensity and duration of stimulation. The individual threads were frequently as much as ten times the length of the animal producing them. Usually, but not always, one end of the Cuvierian organ remained attached to the cloaca. The general appearance and behavior of the threads followed very closely the account given by Minchin (1892) of the discharged Cuvierian organs of H. nigra.

The application of solutions of a variety of "irritating" substances to the surface of H. captiva did not lead to thedischarge of the organs. Half-cubic centimeter volumes of inorganic acids ($\frac{M}{100}$), phenol ($\frac{M}{100}$), turpentine, essential oils, etc., were used. To such solutions the animal reacts vigorously, but not by the expulsion of Cuvierian organs.

A gradual increase in the temperature of the seawater, up to 40°C., did not induce the discharge of the tubes. I have previously noted, in H. surinamensis, the absence of a temperature sense (Crozier 1914a [?]).

Immersion in diluted seawater or in rain water did not lead to Cuvierian organ discharge.

I have elsewhere shown (Crozier, 1914 b) that light has a toxic action on these animals. Holothurians exposed to the direct rays of the sun sometimes discharged a few Cuvierian organs, but after being in bright sunlight for a short time (about 15 minutes) they passed into a moribund condition, in which no tubes were expelled in response to mechanical stimulation. When kept in laboratory aquaria shielded from the light, the partial stagnation of the water was accompanied by the discharge of some of the tubes long before auto-evisceration made its appearance.

It would therefore seem that the normal stimulus for the discharge of these organs is of a tactile or mechanical nature, since it is only in response to such stimuli that the tubes are discharged promptly and elongated to any extent.

III. Mines (1912) found that the discharge of the Cuvierian organs of H. nigra was accompanied by an increase of internal pressure comparable to that which he found sufficient to produce the extension of the isolated tubes, and hence concludes that the tubes are extended by water which is forced into them, in accordance with the view of Hérouard (Delage et Hérouard, 1903). If Holothuria captiva be plunged into water at temperatures between 40°C. and 60°C., the animal contracts somewhat and several Cuvierian organs are usually expelled, though they are coagulated before they are extended beyond a centimeter or so. The internal body pressure may also be artifically raised by pressing on the holothurian's back, in which case the tubes also make their appearance. If the pressure is gentle, they are forced out and break off as small cigar-shaped bodies tapering at both ends, about 8 to 10 mm. long and 0.2 mm. in diameter (fig. 1). By pressing on the animal suddenly and with considerable force the tubes may be caused to be shot out in typical fashion. When placed in rain-water at 36°C., H. captiva dies in less than ten minutes; pressure caused the expulsion of Cuvierian organs from animals killed in this way, but they were never completely extended.

There are a number of facts which indicate that an increase in internal pressure is not the only, nor even the principal factor producing the discharge and elongation of the tubes. When

H. captiva undergoes auto-evisceration,[4] the rupture of the cloacal wall is accompanied by a considerable increase of internal pressure, but it is only rarely that any Cuvierian organs are even partially discharged during this process. Moreover, and this is a factor which has apparently been overlooked, there proceeds from the anus of an animal under experimental pressure, and also in the normal discharge of the organs, a fairly strong current derived from water previously held in the respiratory trees. The direction of this current was observed and its force estimated in water colored with carmine particles. It was clearly evident that the current served to direct and elongate the Cuvierian organs. By this means the organs are generally directed away from the animal discharging them, which is thus prevented from entangling itself. If the unextended organs secured by gentle pressure on a holothurian were taken up in a wide-mouthed pipette and ejected therefrom under the water, they became greatly elongated. A stream of water directed upon an unextended tube lying in a glass dish also produced this elongation, especially when one end of the tube had become attached by its sticky secretion; the latter condition corresponds almost exactly to the state of affairs during the normal discharge of the organs.

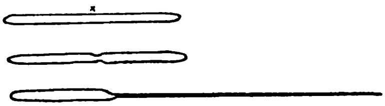

Fig. 1. Showing the reaction of a single Cuvierian organ—obtained by pressing on a Holothuria captiva—to tactile stimulation at the point x. The time occupied by the reaction was in this case 3 seconds. Magnified 2 diameters.

The cloacal stream has also another effect. As is noted by several writers (see Mines, 1912), the Cuvierian organs possess an intrinsic elongating mechanism, consisting of circular muscle fibers and some longitudinal ones. The structure of the tubes of H. captiva agrees very well with Ludwig's account of these organs in H. poli (Ludwig, 1892, p. 173). The short tubes above described as being ejected under gentle pressure may be kept alive for some time in seawater. If they are touched, as with the tip of a fine glass rod, they constrict at about the middle point and from this level proceed to elongate in both directions. Sometimes only the proximal half became constricted and elongated, the distal half—corresponding to the "head" of the organ as described by Minchin (1892) and Russo (1899)—retaining its original form. They also reacted locally by constrictions. Inasmuch as the tubes, as normally discharged, travel faster during part of their course than does the cloacal stream, the water current probably stimulates their extension by the self-contained mechanism. These holothurians when pressed upon sometimes emitted tubes as those above described, which were swirled about by the cloacal stream and stimulated to extension; whereas other tubes of the same sort upon which the cloacal stream did not impinge remained unextended. I therefore believe that the increased body pressure observed by Mines is primarily related to the production of the cloacal stream, and only secondarily implicated in the discharge and elongation of the tubes.

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[4] Even when kept in well aerated seawater aquaria protected from the light, holothurians will eviscerate after about four days. This does not happen, however, until all ingested material has been removed from the gut by defecation. It is therefore possible that one form of the stimulus to evisceration originates in the gut, and it may be suggested that possibly the stimulus results from the autolysis of the intestine, due to the accumulation of digestive enzymes, which under normal conditions are continually removed, since the gut is always full.

There are, then, at least three factors involved in the discharge and extension of the Cuvierian organs; the internal body pressure, the mechanical and stimulating action of the cloacal current, and the intrinsic extending mechanism of the tubes.

The nervous path of the stimuli leading to Cuvierian organ expulsion is not known. With the object of discovering whether the oral nerve ring took part in the matter, I experimented upon animals in which the anterior end had been cut off. The tubes sometimes were ejected by individuals so prepared, but never in a fully extended condition. The result is not decisive, since animals with the anterior end intact frequently presented the same condition after they had been in the laboratory for some time. With such animals, in which the general level of tone is very low, the unextended tubes could be obtained by pressure very much more easily than with the specimens in good condition. This points to the conclusion that the normal release of the Cuvierian organs includes the action of the nervous system, though perhaps in an indirect way.

IV. When the tubes are in the discharged condition they are very sticky. In tubes ejected in water above 40°C. the stickiness is not so evident, even when the tubes are drawn out. Neither are they sticky when extruded into rain water. The adhesive property is somewhat developed when the rain water has been made faintly alkaline. Hence the stickiness depends upon the presence of a substance coagulable by heat, which acquires its adhesiveness through the action of the alkalinity and salts of sea water.

The coelomic fluid of Holothuria is practically sea water. Why, then, do not the tubes stick together as they lie in close contact in the body cavity? It has been generally observed that the tubes are adhesive only when extended. As a matter of fact, the stickiness increases in the slightly extended tubes after they have been for some minutes in seawater. But it would seem that in the usual rapid elongation of the organs the adhesive material is probably squeezed out of gland cells by the contraction of the wall of the tube— a conclusion which is favored by the structure of the organ.

V. SUMMARY

1. The Cuvierian organs of Holothuria captiva are expelled in response to mechanical stimulation, but not to various forms of chemical irritation.
2. The mechanism of their discharge comprises an intrinsic extending apparatus acting together with the stimulating action and directive influence of the cloacal current. The Cuvierian organs are not expelled by the forcing into them of water from the cloaca.
3. The adhesiveness of the discharged Cuvierian organs depends upon the presence of a heat-coagulable substance, which is altered in such a way as to become sticky when it is acted upon by the alkalinity and salts of sea water.

REFERENCES

CLARK, H. L. 1901. Bermudan echinoderms. Proc. Bost. Soc. Nat. Hist., vol. 29, no. 16, pp. 339–345.

CROZIER, W. J. 1914 a (?). The sensory reactions of Holothuria surinamensis Ludw. Zool. Jahrb., Abt. f. Physiol. (in press).

CROZIER, W. J. 1914 b. The orientation of a holothurian by light.. Amer. Jour. Physiol., vol. 36, pp. 1-13, 6 figs.

DELAGE, Y., et HÉROUARD, E. 1903. Les échinodermes. Traité de Zool. concrète, T. 3, x + 495 p., 565 fig., 53 pls.

LUDWIG, H. 1892. Die Seewalzen. Bronn's Thier-Reich, Bd. 2, Abt. 3, Buch I, vi + 460 p., 25 figs., 17 pls.

MINCHIN, E. A. 1892. Notes on the Cuvierian organs of Holothuria nigra. Ann. Mag. Nat. Hist., Ser. 6, vol. 10, pp. 273–284, pl. 17.

MINES, G. R. 1912. Note on the mechanism of discharge of the Cuvierian organs of Holothuria nigra. Quart. Jour. Micr. Sci., vol. 57, pp. 301–304, pl. 26.

RUSSO, A. 1899. Sur valore morfologico e funzionale degli organi di Cuvier delle Oloturie. Monit. Zool. Ital., anno 10, pp. 225–228, 2 fig.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–348, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. Blackman. M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pl. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238. 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pl. July, 1911.
22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., 48 (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., 23 (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., 48 (20) : 737–773. 4 pl. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., 49 (10) : 569–626. 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., 37 (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., 47 (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. 16 (3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., 36 (1) : 8–20. Dec., 1914.
35. Crozier, M. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., 49 (577) : 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of the Holothuria captiva Ludw. Amer. Jour. of Physiol., 36 (2) : 196–202. Jan., 1915.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole, L. J.**—*Pycnogonida* collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
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4. **Coe, W. R.**—The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
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9. **Congdon, E. D.**—The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. **Blackman, M. W.**—The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pl. Feb., 1907.

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19. **Smallwood, W. M., and Rogers, C. G.**—Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8-10): 226–232. Apr., 1910.
20. **Carpenter, F. W.**—Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
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23. **Gross, A. O.**—Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. **Smallwood, W. M.**—Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

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26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23** (4) : 625-636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737-773. 4 pl. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569-626. 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113-130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491-492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443-445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8-20. Dec., 1914.
35. Crozier, M. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28-36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of the Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196-202. Jan., 1915.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
FOR RESEARCH.

No. 38.

ON THE DEVELOPMENT OF THE CORAL AGARICIA FRAGILIS DANA

By J. W. Mavor.

From the Proceedings of the American Academy of Arts and Sciences,
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With Six Plates.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE.— No. 264.

ON THE DEVELOPMENT OF THE CORAL *AGARICIA FRAGILIS* DANA.[1]

By J. W. Mavor.

Presented by E. L. Mark. Received June 15, 1915.

CONTENTS

PAGE.
Introduction 486
Part I. On the larval development 487
  1. General considerations 487
    a. Breeding season 487
    b. Extrusion of the larvae 487
    c. Form of the larva 487
    d. Fixation 488
  2. Anatomy 489
    a. Material, methods and general features 489
    b. Description of the mesenteries, mesenterial filaments and gastrovascular cavities of the larvae studied 492
      Group I. Larva A 492
      " II. Larva B 493
      " " Larva C 494
      " " Larva D 495
      " " Larva E 496
      " III. Larva F 497
      " " Larva G 499
      " " Larva H 500
      " IV. Larva I 501
    c. Conclusions as to the course of development of mesenteries, etc. 504
Part II. On the postlarval development 506
  1. Form of the young polyp 506
  2. The early development of the skeleton 506
    a. Description of skeleton A 506
    b. Description of skeleton B 508
    c. Conclusions on the early development of the skeleton 508
    d. Stage with twelve primary septa 509
  3. Comparison of the development of the skeleton in *Agaricia fragilis* with that in other Hexacorallidae. 509
    Bibliography 510
    Description of plates 511

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[1] Contributions from the Bermuda Biological Station for Research. No. 38.

1. INTRODUCTION.

Interest has been re-awakened of recent years in the development of the *Hexacorallidae* by the papers of Duerden (:04), who has established the order of development of the mesenteries in the larva and of the septa in the attached polyp. As regards the larval development his work agrees in the main with that of Wilson ('88) on *Manicina areolata.* In the skeleton Duerden is the first to find bilateral symmetry in the order of development of the primary exosepta. Our knowledge of the development of coral larvae is in the main confined to four species; *Astroides calycularis* (Lacaze-Duthiers '73); *Manicina areolata* (Wilson '88); *Caryophyllia cyathus* (Von Koch '97) and *Siderastrea radians* (Duerden :04). The early development of the skeleton has been studied in *Astroides calycularis* by Lacaze-Duthiers ('73), in *Caryophyllia cyathus* by Von Koch ('97), in *Caryophyllia clavus, C. smithii* and *Balanophyllia regia* by Lacaze-Duthiers ('97) and in *Siderastrea radians* by Duerden (:04).

In 1907 the writer went to the Bermuda Biological Station for the purpose of studying the development of a recent coral.[2] He was fortunate enough to find the common "hat" or "shade" coral, *Agaricia fragilis,* breeding and was able to rear the larvae.

As the development of the primary mesenteries of the larvae of corals has been worked out in comparatively few cases and a study of the soft parts seemed a necessary prelude to a study of the skeleton, free swimming larvae have been studied both in the living state and in paraffin sections. These observations form the first part of the paper. The writer was not very successful in rearing the young polyps. In consequence only a few skeletons were obtained. The second part of the paper is devoted to a description and discussion of these young skeletons.

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[2] The writer wishes to express his indebtedness to Dr. E. L. Mark for kind assistance and criticism while at the Biological Station and later in the Zoölogical Laboratory of Harvard University.

PART I. ON THE LARVAL DEVELOPMENT.

1. General Considerations.

a. *Breeding Season.*

The first larvae of *Agaricia fragilis* were obtained from a colony of the coral collected in a cave on the shore of Agar's Island, Bermuda, on July 8th, 1907. The colony, which was about eight or nine centimeters in diameter, was brought to the laboratory in the morning and placed in fresh sea water in a battery jar. In the afternoon numerous pear-shaped, light brown, larvae were seen swimming about in the water. Other colonies collected from a cave on Tucker's Island on July 15th when placed in fresh sea water in the laboratory gave off similar larvae. In 1908 larvae were obtained from seven out of eleven colonies over 5 cm. in diameter collected from Long Island, Bermuda, between the 22nd and 30th of June. No larvae were obtained from nine colonies under 5 cm. in diameter collected on June 21st and 22nd from the same place.

These observations show that *Agaricia fragilis* may be found breeding at the Bermuda Islands during the latter part of June and the first part of July.

b. *Extrusion of the Larvae.*

For the purpose of obtaining the larvae, adult colonies of *Agaricia fragilis* were collected in caves and brought to the laboratory in battery jars. During the transference to the laboratory it is probable that in many cases the temperature of the water containing the coral was raised above the temperature of the water in the cave. Larvae were often extruded in large numbers while the corals were being transported to the laboratory and during the few hours immediately succeeding this. What the factors were which produced their extrusion was not determined. Usually, however, not all of the larvae were extruded at this time, a certain number being seen to remain within the parent colony.

c. *Form of the Larva.*

The planula, which is light brown in color, is capable of considerable change of form. It may, however, be described as piriform, the broader, rounded end being aboral and in advance during swimming, while the oral opening is situated at the more pointed posterior end. The alterations in form may be of two kinds; first, elongation, with corresponding decrease in the radial axis, or shortening, with corresponding increase in the radial axis; or, secondly, a contraction of one side of the planula so that the aboral end, which in such cases is usually somewhat flattened, becomes turned to one side. In *Siderastrea radians* Duerden (:04) found the broad rounded end of the larva to be the oral end.

The elongated form is the one usually assumed by the larva when swimming rapidly through the water. The larva takes a more definitely piriform shape when it swims slowly over the substratum. In the latter case there may be a slight in-pushing of the aboral end forming a hollow in the center. This hollowing out of the center suggests a mechanism working by suction. When the larva comes to rest and applies itself to the substratum, it becomes hemispherical, the aboral end being the flat side of the hemisphere and applied to the substratum. Later these larvae may become almost disk shaped. In the preserved specimens the shape is usually either piriform or almost hemispherical. After having become hemispherical and applied to the substratum, the planula may detach itself, elongate and swim away.

d. Fixation.

The planula swims with the broader, rounded, aboral end foremost, as already stated, rotating on its longitudinal axis as it does so. In *Siderastrea radians*, which has the mouth at the broader end of its piriform larva, Duerden (:04) found that, as is the case in *Agaricia fragilis*, the aboral end is kept in front. From this it would seem that while the form of the larva of Siderastraea is favorable for locomotion that of *Agaricia* must satisfy other conditions than that of offering the least resistance to forward motion.

Larvae after they are expelled from the parent colony are usually elongated in shape and swim through the water rapidly. Later they become shorter and broader and swim more slowly. The normal course seems to be for them to affix themselves to the substratum within a few hours after they have been extruded. In the laboratory larvae which did not become fixed in about twenty-four hours after extrusion did not do so when kept for seven days, although during this time they continued to swim through the water and also, as flat disks, to move over the substratum with the aboral end downwards. The failure of such larvae to become attached may have been due to the surface of the glass vessel being unsuitable for fixation. As, however, some of the larvae became fixed on glass while others did not attach themselves even to rough surfaces, such as stones placed in the vessel, the nature of the substratum does not seem to have been the only factor involved. The experiment was tried of keeping larvae in the dark and also under additional pressure,—eighteen inches of water,— but in both cases they failed to attach themselves.

When the larva is about to fix itself it becomes flattened and its aboral end is applied to the substratum. If a stream of water from a pipette be forced against such a larva, the animal may be made to elongate its body again, provided it has not begun the formation of a skeleton. Such larvae may remain in the current of the pipette, appearing as if attached to the surface of the glass by an elastic strand. This suggests that an adhesive mucous substance may be secreted at the aboral end when it becomes applied to the glass. In the free-swimming piriform larvae, especially when they are swimming close to the substratum and apparently in a condition to affix themselves, a concavity may be seen at the aboral end, giving that end the appearance of a suction disk.

Sometimes larvae flattened themselves out into disks at the surface of the water. Such larvae never attached themselves to the bottom or sides of the glass jar and almost all the individuals of such lots became flattened out under the surface of the water. Such larvae tended to fuse into "aggregations" (Duerden :04) and also to go to the sides of the vessel (surface tension). Many of these larvae lived to secrete a skeleton with six well developed primary septa while still floating at the surface of the water.

2. Anatomy.

a. Material, Methods and General Features.

A considerable number of larvae, fixed in various mixtures, were embedded in paraffin and sectioned either transversely or parallel to the oral-aboral axis. Transverse sections were found to be by far the most suitable for the study of the general structure. Nine of the larvae so sectioned have been selected for detailed description in order that the reader may have in as concrete a form as possible the data on which the conclusions of the paper are based. Although only individual differences have been observed between the opposite sides of the bilaterally symmetrical planulae, it has been thought best for convenience in description to distinguish a right and a left side. In this connection the aboral end of the planula, which is foremost in locomotion, has been considered anterior while the oral end is considered posterior.

Each larva was cut into a series of transverse sections of equal thickness, which varied from 5 μ to 7 μ for the different series. In recording the position of structures in the larvae the thickness of the sections has been used as a unit of measure.

The nine larvae fall into four groups:— First, larva A, which has four pairs of mesenteries, only two of which are well developed; Second, larvae B, C, D, and E, in which there are six pairs of mesenteries, the fifth and sixth however being only slightly developed and the mesenterial filaments not extending the whole length of the larva; Third, larvae F, G and H, in which all six mesenteries are well developed and the mesenterial filaments of the first two pairs of mesenteries extend through the greater part of the larvae; Fourth, larva I, in which, besides the greater development of the fifth and sixth pairs of mesenteries, there are developed mesenterial filaments on the third pair of mesenteries.

The musculature of the mesenteries consists of fibres developed in endoderm cells where these abut on the mesogloea. These fibres stain deeply with haematoxylin and so are easily distinguished in the preparations. In the mesenteries the majority of these fibres run longitudinally. Some, however, especially those near the junction of the mesogloea of the mesentery with that of the body wall, run obliquely or even transversely. It is not always easy to determine which side of the mesentery shows the large number of fibres: first, because the mesogloea in the mesenteries is usually very thin and does not take stain well, and, secondly, because there are always some fibres on each side. This is still more difficult in the newly formed mesenteries, as in these there is almost always an approximately equal number of fibres on each side of the mesentery. The distribution of the muscle fibres given in the diagram (text Fig. 1, *C*) is based on careful examination with a 2-mm. apochromatic objective and compensating oculars x8 and x12. In the first and second pairs of mesenteries the muscle fibres are confined almost entirely to the ventral sides. In the third and fourth pairs, the directives, the muscle fibres are more numerous on the lateral sides. In the fifth and sixth pairs, when a difference can be observed between the two sides of the mesenteries, the fibres are most numerous on the dorsal side (Fig. 2, *E*). In the mesenteries in which a mesenterial filament is developed (first and second pair), the muscle fibres are most numerous in that region of the mesentery which is about twice as far from the body wall as from the internal margin of the mesentery, in those sections in which the filament appears. In all other cases the muscle fibres are most numerous along the internal edges of the mesenteries and are usually numerous on both sides of the mesentery along this edge.

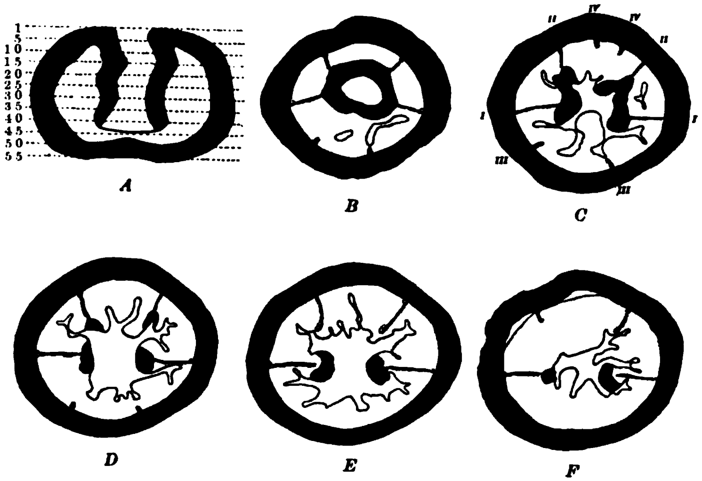


FIGURE 1. *Agaricia fragilis.* Larva A. *A*, a longitudinal section through the first pair of mesenteries and their filaments, reconstructed from transverse sections. Viewed from the dorsal side. The numbers, and the dotted lines corresponding to them, indicate the numbers and positions of the transverse sections of the series. Ectoderm and mesoderm black. *B*, *C*, *D*, *E*, and *F* represent sections number 14, 18, 23, 30 and 41, respectively. The reader views the aboral face of the section, and dorsal is up, so that the right of the larva is on the left of the figure. In *C*, *I*, *II*, *III*, *IV* indicate the first, second, third and fourth pairs of mesenteries, respectively. × 90.

The musculature of the body wall consists of a layer of longitudinal fibres developed in the inner ends of the ectoderm cells where they abut on the mesogloea and of a layer of circular muscles developed in the endoderm cells where they reach the mesogloea of the body wall. The mesoderm of the body wall, although thin, is everywhere clearly to be seen.

At the oral end the ectoderm of the body wall is folded in to form the oesophagus and the filaments of the first two pairs of mesenteries. At its oral end the oesophagus forms a complete tube. Aborally it becomes divided dorsally so that it extends for some distance as a scoop-shaped structure, U-shaped in cross section. In the following descriptions the point where the oesophagus ceases to be a complete tube and becomes scoop-shaped is expressed by saying that it is interrupted dorsally.

b. Description of the Mesenteries, Mesenterial Filaments and Gastrovascular Cavities of the Larvae Studied.

Group I. Larva A.

In this larva (text, Fig. 1) the first two pairs of primary mesenteries are well developed and there are indications of the third and fourth pairs. The larva was fixed in Flemming's chromo-aceto-osmic mixture and the sections were stained with Heidenhain's iron haematoxylin. The aboral end of the larva was sectioned first and the sections are almost exactly transverse, the ventral side of the oral end, however, extends six sections beyond the dorsal side, a deviation from the transverse which cannot be detected at the aboral end. The sections are 7 micra thick and there are 56 of them in all. Text-figure 1 shows at *A*, a reconstructed longitudinal section passing through the mesenterial filaments of the first pair of mesenteries. The horizontal dotted lines are drawn in the planes of sections and each of the numbers at the side of the figure indicates the number of the section to which the line corresponds, beginning with the oral end. The figure shows that the aboral end of the larva is flattened and contains a slight concavity. This shape may be due to contraction on the application of the fixing fluid, but larvae of this form were observed in the living state. At *B*, *C*, *D*, *E*, and *F*, in Figure 1, are shown the 14th, 18th, 23rd, 30th, and 41st sections respectively from the oral end. Photomicrographs of sections 15, 24, 30, and 46 are shown in Plates 1 and 2.

The oesophagus is irregularly circular in cross section (Fig. 1, *B*) and extends aborally in the axis of the larva. The fact that it appears nearer to the dorsal side in the figure is due to the section having been cut somewhat obliquely. The larva has been cut so that the oesophagus appears as a complete tube first in section 6, the ventral side only appearing in the earlier sections. In section 8 the endoderm appears on the ventral side and in section 14 on the dorsal side for the first time. The oesophagus is interrupted first ventrally in section 16 and then dorsally in section 19. In the sections the oesophagus is cut obliquely on the ventral side while on the dorsal side it is sectioned transversely. In spite of this the oesophagus extends on the ventral side through 12 sections, while on the dorsal side it extends through only 6 sections.

Although interrupted on the ventral side, the ectoderm of the oesophagus is continuous with the ectoderm of the mesenterial filaments of the first two pairs of mesenteries (Fig. 1, *C*). The mesenterial filaments of mesenteries *I* and *II* of the right side of the larva, (left side of the figure) are fused together as far as section 19, after which they become separated; those of the left side are fused as far as section 21. The mesenterial filaments of the first pair of mesenteries extend on the right side of the larva as far as section 43; on the left side of the larva as far as section 41. They are larger than the filaments of the second pair of primary mesenteries and there is no evidence of an aboral enlargement as found in the larvae of the later stages. The mesenterial filaments of the second pair of mesenteries extend on the left side into section 28 and on the right side into section 34. The mesenteries of the first pair extend on the right side of the larva from section 9 to 46, and on the left side from section 11 to 48. Those of the second pair extend on the right from section 13 to 46, on the left from section 14 to 46. The beginning of the development of the third pair of mesenteries appears on the right of the larva between sections 8 and 31 and on the left between sections 10 and 27. At their oral ends the third pair of mesenteries extends to the ectoderm of the oesophagus. Slight indications of the fourth pair of mesenteries are found on either side in sections 12 to 18 (Fig. 1, *C*). Here also the mesentery as defined by mesoglea and muscles extends to the ectoderm of the oesophagus. The distance to which the muscles and mesoglea of the fourth pair of primary mesenteries extend into the endoderm is less than in the third pair.

Group II. Larva B.

This larva has six pairs of mesenteries all clearly shown. It was fixed in a saturated solution of corrosive sublimate to which 1% acetic acid was added, and the sections were stained in Heidenhain's iron haematoxylin and congo red. The sections deviate very slightly from the transverse, the right dorsal side of the oral end of the larva being cut first. As the oral end was cut first the right side of the larva is to the right in the preparation. The sections are 7 μ in thickness and there are 57 of them in all. As in the previous larva, the aboral end is somewhat flattened.

The oesophagus is circular in cross section, and extends on the ventral side through one more section than on the dorsal side. On the ventral side it extends into section 22. After leaving their junction with the oesophagus in section 22 the mesenterial filaments of the first and second mesenteries of either side are not fused as in larva A. Those of the first pair of mesenteries extend on either side into section 42 and those of the second pair are turned from the aboral end of the oesophagus outwards toward the oral end of the larva extending on the right into section 12 and on the left into section 17. The filaments of the first pair of mesenteries are enlarged on the right side between sections 30 and 37, and on the left side between sections 37 and 39, those of the second pair of mesenteries decrease gradually in size until they end. Only mesenteries *I* and *II* are complete.

The lengths and positions of the mesenteries are given in the following table, which shows the number of the section where each mesentery begins and ends:

Number of mesentery	*I*	*II*	*III*	*IV*	*V*	*VI*
Right side of larvae						
Oral end section No	7	9	10	11	8	7
Aboral end section No.	45	38	44	36	44	31
Left side of larvae						
Oral end section No.	11	11	12	11	12	12
Aboral end section No.	48	40	44	36	44	36

Although traces of the fifth pair of mesenteries extend into section 44 these mesenteries are only slightly developed in the aboral half of the larva.

Group II. Larva C.

This larva is in about the same stage of development as larva B, but the fifth and sixth pairs of mesenteries show more clearly. It was fixed in a saturated solution of corrosive sublimate plus 1% acetic acid. The 90 sections were stained with Heidenhain's iron haematoxylin and congo red, each being 5 μ thick. The aboral end of the larva was cut first and on the dorsal side, so that the ventral side of the oral end occurs in nine sections after the dorsal side has ceased to appear.

The oesophagus is almost round in cross section and is shorter on the dorsal side than on the ventral side, the length of the dorsal side being approximately 50 μ, while that of the ventral side is 170 μ.

The mesenterial filaments of the first and second pairs of mesenteries are continuous with the ectoderm of the oesophagus. On the right side they are fused in section 24 but below that separate, the filament of the first mesentery extending into section 64, that of the second into section 57. On the left side they are fused as far as section 45, below which the filament of the first mesentery extends as far as section 63; the second mesentery has no separate mesenterial filament. The filaments of the first pair of mesenteries are enlarged and crescent shaped in cross section on the right between sections 43 and 61 and on the left between sections 40 and 59. The filaments of the second pair of mesenteries are not thus enlarged and are approximately uniform in cross section throughout their length.

The lengths and positions of the mesenteries are given in the following table:

Number of mesentery	*I*	*II*	*III*	*IV*	*V*	*VI*
Right side of larva						
Oral end section No.	15	16	7	20	12	20
Aboral end section No.	69	74	55	82	47	66
Left side of larva						
Oral end section No.	11	16	7	20	8	21
Aboral end section No.	68	74	55	82	47	66

Only mesenteries *I* and *II* are complete.

Group II. Larva D.

Larva D, which was in nearly the same stage of development as larvae B and C, was fixed in a saturated solution of corrosive sublimate, and the sections were stained in Mallory's phosphotungistic acid haematoxylin. The plane of sectioning deviated slightly from the transverse, the first part of the larva cut being slightly dorsal to the middle of the right side. The 47 sections are each 7 μ thick.

The oesophagus is elliptical in cross section, the long axis being dorso-ventral.

The mesenterial filaments of the first and second pairs of mesenteries of the right side are not fused together after they leave the oesophagus. The first of these extends from sections 13 to 26, the second from sections 13 to 25. On the left side the filaments are fused from their junction with the oesophagus at section 13 as far as section 17, below which the filament of the first mesentery extends to section 29, that of the second to section 25. The filaments of the mesenteries are enlarged as follows: those of the first pair on the right between sections 19 and 22, on the left between sections 18 and 23, those of the second pair on the right between sections 19 and 23, on the left between sections 18 and 21.

The length and position of the mesenteries is shown in the following table:

Number of mesentery	*I*	*II*	*III*	*IV*	*V*	*VI*
Right side of larva						
Oral end section No.	7	6	9	6	14	5
Aboral end section No.	35	31	38	34	37	34
Left side of larva						
Oral end section No.	9	10	9	7	13	18
Aboral end section No.	44	39	40	34	42	38

Only mesenteries *I*, *II* and *III* are complete.

Group II. Larva E.

This larva was fixed and stained like larva D. The aboral end was cut first and the sections are so nearly transverse that all sides of the circular oesophagus appear in the next to the last section of the series. There are 55 sections, each 7 μ thick and they are numbered from the *oral* end.

The oesophagus is elliptical in cross section. Its dorsal side ends at section 7 and its ventral side at section 11.

The mesenterial filaments of the first and second pairs of mesenteries are separate where they leave the oesophagus. The filaments of the first pair extend on either side from the oesophagus, at section 11, to section 47. Those of the second pair extend on the right to section 31 and on the left to section 35. The filaments of the first pair are enlarged in cross section on both sides between sections 23 and 44. Those of the second pair on the right between sections 16 and 29 and on the left between sections 16 and 34.

The position and extent of the mesenteries is shown in the following table:

Number of mesentery	*I*	*II*	*III*	*IV*	*V*	*VI*
Right side of larva						
Oral end section No.	4	4	5	5	6	6
Aboral end section No.	49	49	49	44	49	44
Left side of larva						
Oral end section No.	6	6	5	5	9	9
Aboral end section No.	50	49	51	44	50	44

In section 49 mesenteries *I* and *II* of the right side are united at their internal ends. Only mesenteries *I* to *IV* are complete.

Group III. Larva F.

This larva (text, Fig. 2) is not so much contracted along its oral-aboral axis as the one last described, and the oesophagus is only slightly invaginated. It was fixed in a saturated solution of corrosive sublimate and the sections stained in Mallory's phosphotungstic acid haematoxylin. The oral end of the larva was cut first. The sections are almost exactly transverse. The oesophagus appears as a complete circle of ectoderm in the third section, the first two sections showing the dorsal side only. The sections are 6 μ thick and there are 86 of them. Of the sections, Figure 2, *A–I*, shows numbers 9, 13, 18, 21, 33, 45, 61, 71 and 74 and Plates 3 and 4 show photomicrographs of numbers 12, 17, 61 and 73.

The oesophagus is elliptical in cross section, the long axis being dorso-ventral. It ceases on the dorsal side at section 9 (Fig. 2, *A*). Below this its cross section is U-shaped until section 13 (Fig. 2, *B*) is reached. Below section 13 it is interrupted on the ventral side also. The invaginated ectoderm of the right side of the larva is joined by the first, second and third mesenteries, that of the left side is joined by the first and second mesenteries (Fig. 2, *C*).

Below section 20 the ectoderm is continued on both sides as the separate mesenterial filaments of the first and second pairs of mesenteries. The filaments of the first pair extend on the right as far as section 70 and on the left as far as section 73, which is practically the whole extent of the gastrovascular cavity. The filaments of the second pair extend on both sides as far as section 56. The mesenterial filaments are enlarged as follows: those of the first pair of mesenteries on the right between sections 49 and 66, on the left between sections 52 and 70, those of the second pair of mesenteries on both sides between sections 32 and 54 (Fig. 2, *E–H*).

The six pairs of primary mesenteries extend practically the whole length of the endoderm of the larva. The following table shows their positions and extent:

Number of mesentery	*I*	*II*	*III*	*IV*	*V*	*VI*
Right side of larva						
Oral end section No.	8	8	9	8	9	13
Aboral end section No.	75	75	72	70	72	75
Left side of larva						
Oral end section No.	9	8	9	8	10	12
Aboral end section No.	77	76	72	70	72	77

Only mesenteries *I* to *IV* are complete.

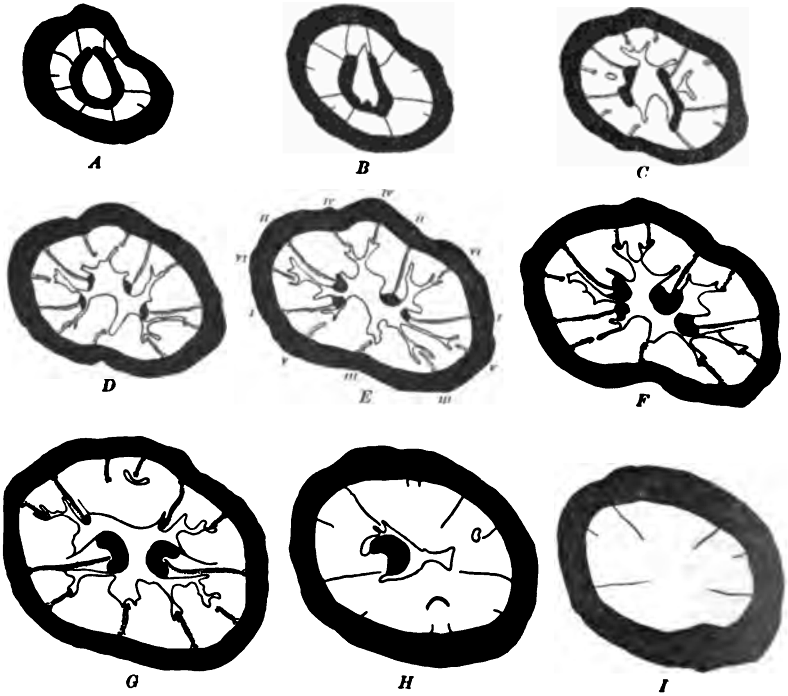


FIGURE 2. *Agaricia fragilis*. Larva F. Transverse sections the oral end having been cut first. The right of the larva is to the right in the figures. Ectoderm and mesoglea colored black. *A, B, C, D, E, F, G, H, I* sections number, 9, 13, 18, 21, 33, 45, 61, 71, and 74, respectively. In *E*, *I*, *II*, *III*, *IV*, *V* and *VI* mark the first, second, third, fourth, fifth, and sixth sections. × 90.

Group III. Larva G.

The chief differences between this larva and larva F pertain to the form of the oesophagus and gastrovascular cavity. It was fixed in Flemming's fluid and stained with Heidenhain's iron haematoxylin. The series consists of 154 transverse sections each 7 μ thick. The aboral end was cut first, so that the sections are viewed from this end, but the numbering of the sections is from the oral end.

The oesophagus is well invaginated and is circular or slightly oval in cross section (Fig. 3, *A*) as far as section 20. It is then interrupted on the dorsal side, as seen in sections 21–23 (Fig. 3, *B*), after which the ectoderm becomes divided also on the ventral side. The slight deviation of the sections from the true transverse plane is such that their ventral edges are more aboral than their dorsal edges, a fact which can be learned from the table showing the extent of the mesenteries, and from the fact that the last few sections cut the ventral side only of the larva. There can be no doubt, therefore, that the oesophagus is interrupted on the dorsal before it is on the ventral side.

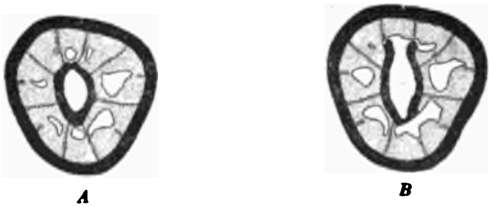


FIGURE 3. *Agaricia fragilis.* Larva G. Transverse sections, aboral aspect. Ectoderm and mesoglea colored black. *A*, section 19; *B*, section 23. × 90.

There are only two pairs of mesenterial filaments, those of the first and second pairs of mesenteries. The filaments of the first and second mesenteries are fused on the left from sections 24 to 31 and on the right from sections 24 to 33. The sections of the filaments of the first pair of mesenteries decrease in size until section 57 is reached, after which they increase in size on both sides up to about section 124, where they are somewhat larger than those of the second pair of mesenteries. After this they decrease in size till they end, on the right at section 135 and on the left at section 132. The filaments of the second pair of mesenteries increase in size up to about section 57. In section 40 they have about the same size as those of the first pair of mesenteries

mesenteries as far as section 80 on the right and on the left up to section 83. They are largest (Fig. 4, *F*) on the right between sections 87 and 108 and on the left between sections 87 and 110, where they are larger than any of the transverse sections of the filaments of the second pair of mesenteries. The mesenterial filaments of the second pair of mesenteries extend on the right into section 89 and on the left (Fig. 4, *F*) into section 95. The transverse sections of these filaments (Fig. 4, *E*) are largest on the right between sections 54 and 87 — where up to section 80 they are larger than the filaments of the first pair of mesenteries in the same sections,— and on the left between sections 58 and 90, where they are larger than the filaments of the first pair of mesenteries as far as section 83. The filament of mesentery *III* on the right side of the larva (left in the preparation) is not continuous with the ectoderm of the oesophagus, being absent in sections 20 to 26. On the left side of the larva the filament of the corresponding mesentery if present at all is reduced to a few scattered cells in these sections. Both filaments are enlarged beyond these sections but are always much

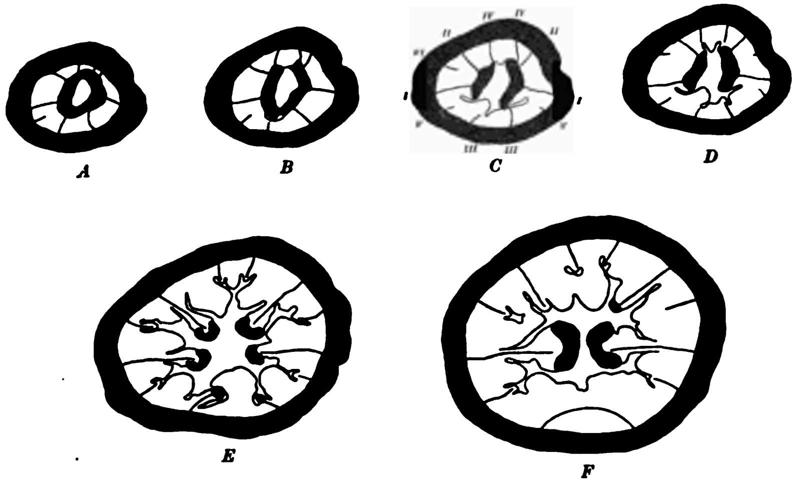


FIGURE 4. *Agaricia fragilis*. Larva I. Transverse sections of larva, the oral end having been cut first. Dorsal being up, the right of the larva is to the right in the figures. Ectoderm and mesoglea colored black. *A, B, C, D, E, F*, sections number 15, 19, 23, 25, 73, 93 respectively. In *C*, *I, II, III, IV, V, VI* mark the first, second, third, fourth, fifth, and sixth pairs of mesenteries. × 90.

smaller than the filaments of mesenteries *I* and *II*. The filament on the left of the larva (right in the sections) extends to about section 73 (Fig. 4, *E*), that on the right of the larva to about section 77, the limits not being sharply defined.

The filaments of mesenteries *III* differ histologically from those of *I* and *II*. The ciliated cells are shorter and thicker and gland cells seem to be absent, resembling in this the endoderm cells, from which the filament is not always sharply to be distinguished.

A table is given showing the extent of the six primary mesenteries in this larva.

Number of mesentery	*I*	*II*	*III*	*IV*	*V*	*VI*
Right side of larva						
Oral end section No.	9	12	9	15	13	23
Aboral end section No.	115	112	102	104	112	102
Left side of larva						
Oral end section No.	12	15	11	15	21	26
Aboral end section No.	115	112	102	104	112	102

Only mesenteries *I* to *IV* are complete.

This table shows that all of the mesenteries extend practically the whole length of the larva. The third and fourth mesenteries are, however, considering their early appearance in development, somewhat shorter than the others. The mesoglea of the mesenteries is very thin and in many cases can be seen only where it is cut obliquely. That it is somewhat tough and rigid, at least in fixed and stained preparations, is shown by its being slightly displaced in sections, leaving the less resistant entoderm cells behind it.

The gastrovascular cavity is clearly in the process of being divided into compartments corresponding to the mesenteries. It will be noticed in Figure 4, *E* and *F*, that a cleft in the entoderm occurs on either side of each mesentery and that entoderm bulges inward between the mesenteries and between these clefts. This would seem to indicate that the formation of the compartments is brought about by the formation of the mesenteries. There is evidence in the histology of the entoderm that the clefts referred to are formed by the separation and breaking down of cells rather than by a process of infolding. This would tend to support the theory that the clefts are due to the closer adherence of the contiguous entoderm cells to the more rigid mesoglea of the mesenteries.

c. *Conclusions as to the Course of Development of the Mesenteries, Mesenterial Filaments and the Gastrovascular Cavity.*

If the assumption be made that the mesenteries develop in the order of their size, the order of their development in the larva of *Agaricia fragilis* is indicated by the numbers given the mesenteries (Fig. 2, *E*), with the exception, that numbers V and VI develop simultaneously. So far as the first four pairs of mesenteries are concerned the order of development is that found by Faurot ('95) in *Adamsia palliata* and *Halcampa chrysanthellum;* by Wilson ('88) in *Manicina areolata;* by McMurrich ('91) in *Rhodactis sancti-thomae* and *Aulactinia* and by Duerden (:04) in *Siderastrea radians.*

The writer believes that the larvae studied show that in the development of the six pairs of primary mesenteries there may be recognized three periods as follows: first, a period in which there are two pairs (*I* and *II*), a condition not represented by any of the larvae, but shown probably to exist by the great development of pairs *I* and *II* and the only slight indication of pairs *III* and *IV* in larva A; second, a period in which there are four pairs, represented in an early stage by larva A, and shown probably to exist by the large size of pairs *III* and *IV* and the small size of pairs *V* and *VI* in larvae B and C; third, a period in which there are six pairs of mesenteries represented by larvae B to I. By dividing the development of the mesenteries into these periods the writer does not wish to deny the appearance of the pairs of mesenteries in succession, but merely to show their association into three sets of two pairs each. Further, it is to be noticed that this association in sets of two pairs becomes more intimate as development proceeds. In larva A pair *I* shows a very considerable advance over pair *II* and this difference persists to some extent in the older larvae. In the same larva pair *III* shows only a slight advance over pair *IV* and in the older larvae such a difference between pairs *III* and *IV* is hardly to be seen. In larvae B to I pairs *V* and *VI* seem to have appeared and developed simultaneously.

If this interpretation is correct, the ventro-dorsal order of development which is evident in the first two pairs, becomes less marked in the next two pairs (*III* and *IV*) and has entirely disappeared in the last two pairs (*V* and *VI*). In other words the bilateral symmetry of the larval mesenteries has begun to give way to a radial symmetry while it is still free swimming.

Evidence of close association in development between the first two pairs of mesenteries is seen also in the development of their mesenterial filaments, which are already well developed when the third and fourth pairs of mesenteries have only begun to show (larva A). The mesenterial filaments of the third pair of mesenteries show only after the six pairs of primary mesenteries have reached a comparatively advanced stage of development (Larva I).

The bilateral symmetry of the planula is shown in the position, as well as the order of development, of the six pairs of primary mesenteries. This is clearly shown in the position of pairs *I* and *II*. The two mesenteries of pair *I* lie almost in the same straight line in cross sections, while the mesenteries of pair *II* if produced would meet at an angle of about 45 degrees (Fig. 1, *D;* Fig. 2, *I;* Fig. 4, *F*). Further, if the transverse sections be examined, it will be noted that the length of the circumference on the ventral side between the peripheral ends of the mesenteries of pair *I* is greater than the length of the circumference between the peripheral ends of pair *II*. Both these facts may be expressed by saying that the angle between the mesenteries of pair *I* on the ventral side is greater than the angle between the mesenteries of pair *II* on the dorsal side.

The transverse sections show that in the older larvae the oesophagus is elliptical in cross section and that the long axis of the ellipse is dorsoventral, a condition found in *Manicina areolata* by Wilson ('88). The oesophagus is continued further aborally on the ventral than on the dorsal side.

The mesenterial filaments of mesenteries *I* and *II* are continuous with the ectoderm of the oesophagus and are formed by an aboral growth of this ectoderm. These filaments show the same histological structure as the ectoderm of the outer surface, lacking however nettle cells. The condition found in larva I seems to show that the mesenterial filaments of mesenteries *III* are developed from the endoderm without any connection with the ectoderm.

In *Manicina areolata* Wilson ('88) has shown that the filaments of the first three pairs of mesenteries are developed from the oesophageal ectoderm. In *Aulactinia* McMurrich ('91) found that there was no reflection of the ectoderm as described in *Manicina* by Wilson ('88), and that the median streak (which appears before the lateral streaks) of the filaments of the first three mesenteries was developed from the endoderm.

My preparations seem to show that the gastrovascular cavity in Agaricia is developed by a breaking down and splitting of the endoderm and that the mesenteries, muscle cells, and the cells which will form or have formed the mesenterial filaments are the agents which determine its form.

PART II. ON THE POSTLARVAL DEVELOPMENT.

1. Form of the Young Polyp.

At the time of the flattening out of the young polyp soon after fixation there is no evidence of tentacles. The six pairs of primary mesenteries show clearly as grooves on the surface and when viewed by transmitted light as dark lines. The oral aperture, as in the planula, is oval. The young polyps were not studied in sections as it was desired to preserve the skeletons.

2. The Early Development of the Skeleton.

The study of the skeleton has been confined to the skeletons of the larvae which fixed themselves to the glass vessels in which they were kept. In spite of numerous efforts to rear young polyps and to obtain early stages in the development of the skeleton, only a few skeletons suitable for studying the development of the primary ento-septa were obtained. Two of these have been chosen for description.

a. Description of Skeleton A.

This skeleton (Plate 5, top figure) shows a thin calcareous layer, the basal plate, covering the area of the glass to which the polyp has attached itself. This layer is slightly thickened in the centre, apparently an unusual condition; for a columella is not developed in the older skeletons obtained. The six primary entosepta are arranged radially, extending from near the periphery about half way toward the center. The two entosepta on the left and the upper one on the right show forking at their peripheral ends. The basal plate is thickened near the entosepta in areas corresponding to the primary entocoels. A thin outer ring which extended round the basal plate was largely destroyed in the maceration, a small part of it is shown in the lower part of the photograph. It surrounded the outer limit of the soft tissues of the polyp. The opacity of the young polyp made it impossible to study the relations of the skeleton to the soft parts while the animal was still alive. So an outline drawing was made of the living polyp showing the positions of the mouth and mesenteries. When the polyp was killed and the soft parts macerated it was found that this drawing could be fitted with certainty over a drawing of the skeleton (Fig. 5), owing to the exact correspondence between the periphery of the basal plate and the outline of the soft parts of the polyp. The drawings show that the primary entosepta lie between the primary mesenteries and, when compared with the photograph (Plate 5, top figure), that the thickenings of the basal plate referred to above coincide exactly with the areas enclosed in the primary entocoels.

It will be noticed in both photograph and drawing that the primary

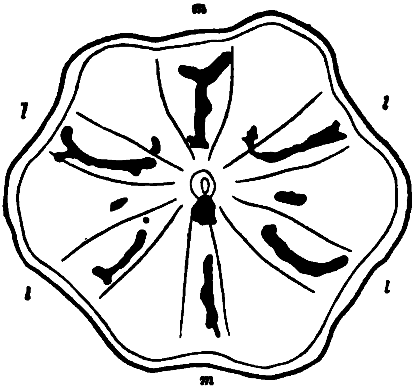


FIGURE 5. *Agaricia fragilis*. Outline drawing of a young polyp, showing the six pairs of primary mesenteries and the opening of the mouth, superposed on a drawing of the skeleton of the same polyp (septa in solid black) made separately after the polyp had died and disintegrated. *m, m,* median septa, *l, l,* lateral septa.

septa show bilateral symmetry in their arrangement and form. Unfortunately it was not possible in the living polyp to distinguish a dorsal from a ventral side. That the axis of this symmetry is, however, dorso-ventral is shown by the oral aperture, which has its long axis in this direction. The axis of symmetry is occupied by two entosepta, which will be called median (Fig. 5, *m*); the upper (in the figure) lateral septa (*l*) make equal angles with the median plane. This is also true of the lower lateral septa, but the angle which the latter make is less than that made by the upper septa. All four of these septa are concave toward the upper side of the figure.

Both photograph and drawing show two pairs of very small exosepta. Owing to ignorance as to which of the median septa is dorsal, it is not possible to say whether the upper pair is the dorso-lateral or ventro-lateral pair. The photographs and drawing have been oriented, however, to correspond with the arrangement found by Duerden (:04) in *Siderastrea radians*, where the dorsal septa are the first to develop.

b. Description of Skeleton B.

In this skeleton a distinct epitheca surrounds the basal plate. The entosepta are relatively larger than those of skeleton A. There is the same bilateral symmetry with regard to the angles which the lateral septa make with the median plane. The lateral septa, however, in this case are slightly concave on the lower side. Exosepta and columella are absent.

c. Conclusions on the Early Development of the Skeleton.

The early skeletons obtained show great variation in size and in the development of the septa. In some cases only five septa are developed, in others one or more septa are defective. This variation may be due to the fixation of the larvae at an earlier period in their development than is usual; the skeletons showing most variation are the smaller ones.

The following conclusions may however be drawn. The basal plate and the six primary entosepta are the first structures to be developed. The primary exosepta do not arise simultaneously. Bilateral symmetry is frequently shown in the arrangement of the primary entosepta.

Four possible explanations of this bilateral symmetry occur to the writer. (1) The polyps which formed these skeletons may have fixed themselves with the dorsal or possibly ventral side bent over toward the substratum. (2) One or more of the median entocoels may have been enlarged by the growth of the wall of the polyp in that region. (3) It may represent a persistence of the bilateral symmetry seen in the development of the mesenteries. (4) It may represent the tendency of the coral to grow upward at one point to form the frond-like corallum of the older coral.

d. Stage with Twelve Primary Septa.

A number of skeletons in this stage were obtained (Plate 5, bottom photograph). They show six primary entosepta and six exosepta. The epitheca covers the outer portion and is in the form of the base of a cone. It is decorated with ridges running toward the oral end of the polyp. The theca is seen joining the entosepta and exosepta. The primary entosepta extend beyond the theca but do not reach the epitheca. In a few of the skeletons a slight indication of the bilateral symmetry described for the earlier skeletons could still be recognized.

3. COMPARISON OF THE DEVELOPMENT OF THE SKELETON IN AGARICIA FRAGILIS WITH THAT IN OTHER HEXACORALLIDAE.

In *Astroides calycularis* Lacaze-Duthiers ('73) found that each of the six primary entosepta was formed from three centres of calcification, the septa being in consequence Y-shaped with the upper part of the Y toward the periphery. The two septa on the left in Plate 5 (top figure) are forked at their peripheral ends, although the prongs of the fork are not so large as in *A. calycularis*. Lacaze-Duthiers found that the other septa appear irregularly. The same species has been studied by Koch ('82), who also finds the primary septa forked. Both these authors find that the primary entosepta are developed before the theca or epitheca.

In *Caryophyllia cyathus* and *C. clavus* Lacaze-Duthiers ('97) found similar stages in the development.

In the development of *Siderastrea radians*, studied by Duerden (:04), six primary entosepta are developed as single continuous rods without connection with the theca or epitheca. An outer ring, the beginning of the epitheca, similar to the ring described in skeleton A, surrounds the basal plate. The primary exosepta are developed in dorso-ventral succession and independently of the entosepta.

UNIVERSITY OF WISCONSIN,
April, 1915.

BIBLIOGRAPHY.

Duerden, J. E.

1904. The Coral Siderastrea radians and its Postlarval Development. Carnegie Institution, Washington. Publn. No. 20. v + 130 p., 11 pl.

Faurot, L.

1895. Études sur l'Anatomie, l'Histologie et le Développement des Actinies. Arch. de Zool. Exp. et Gen., Sér. 3, Tom. 3, p. 43–262, pl. 1–12.

Koch, G. von.

1882. Ueber die Entwicklung des Kalkskeletes von Asteroides calycularis und dessen morphologischer Bedeutung. Mitt. Zool. Sta. Neapel, Bd. III, p. 284–292, Taf. 22–24.

1897. Entwicklung von Caryophyllia cyathus. Mitt. Zool. Sta. Neapel, Bd. XII, p. 755–772, Taf. 34.

Lacaze-Duthiers, H. de.

1873. Développement des Coralliaires. Deuxième Mèmoire. Actiniaires à Polypiers. Arch. de Zool. Exp. et Gen., Tom. II, p. 269–348, pl. 12–14.

1897. Faune du Golfe du Lion. Coralliaires. Zoanthaires Sclérodermés. Arch. de Zool. Exp. et Gen., Sér. 3, Tom. 5, p. 1–249, pl. 1–12.

McMurrich, J.

1891. Contributions on the Morphology of the Actinozoa. II. On the Development of the Hexactiniae. Journ. Morph., Vol. 4, p. 303–330, pl. 13.

Wilson, H. V.

1888. On the Development of Manicina areolata. Journ. Morph., Vol. 2, p. 191–252, pl. 15–20.

DESCRIPTION OF PLATES.

Plates 1–6. Development of *Agaricia fragilis*. All, except top and bottom figures of Plate 5, are photomicrographs of transverse sections of free swimming larvae. × 175.

Plate 1. Larva A, upper photograph, section 15; lower, section 24.

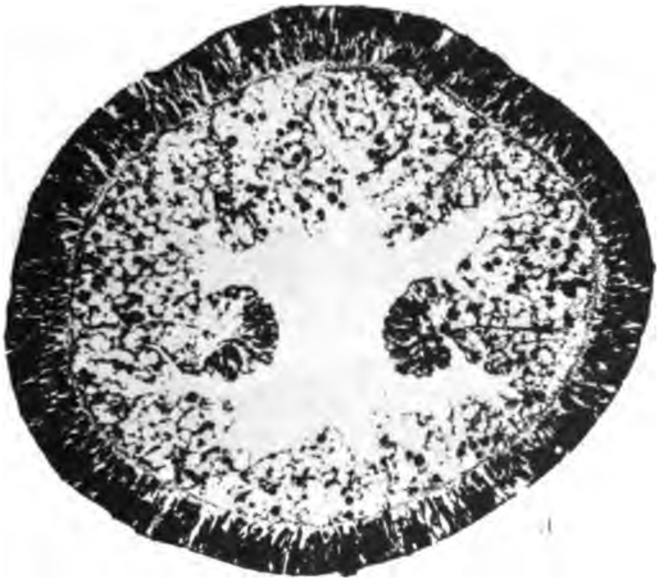
Plate 2. Larva A, upper photograph, section 30; lower, section 46.

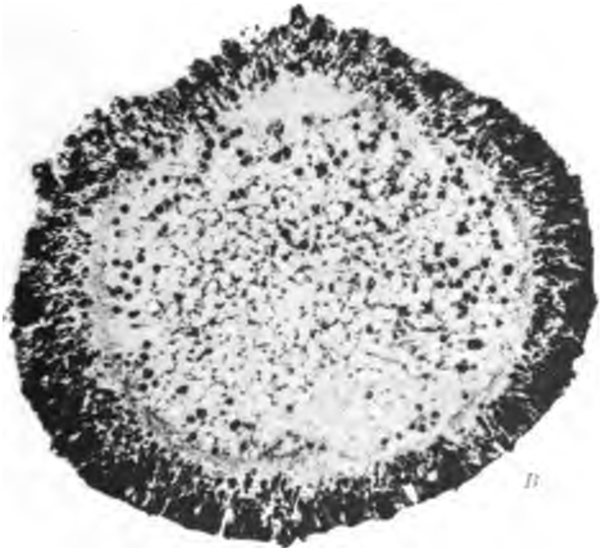
Plate 3. Larva F, upper photograph, section 12; lower, section 17.

Plate 4. Larva F, upper photograph, section 61; lower, section 73.

Plate 5. Two middle figures are of Larva I, the left (*C*) being section 19; the right (*D*), section 26. The top figure (*A*) is a photograph of skeleton A, the bottom one (*B*) of skeleton C.

Plate 6. Larva I, upper photograph, section 73; lower, section 93.





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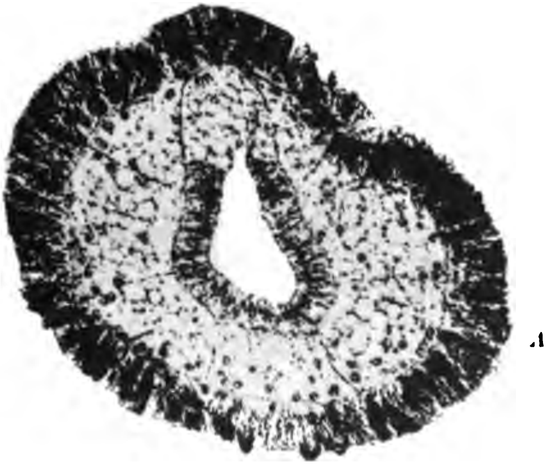

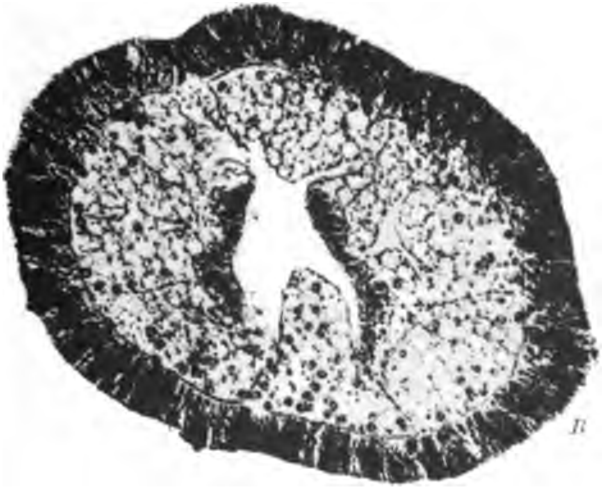

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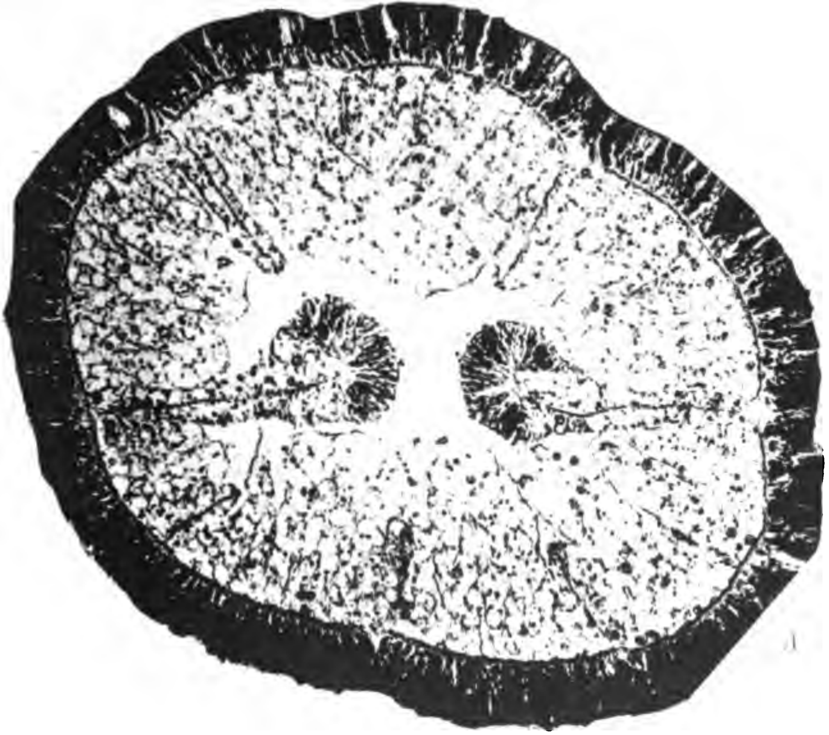

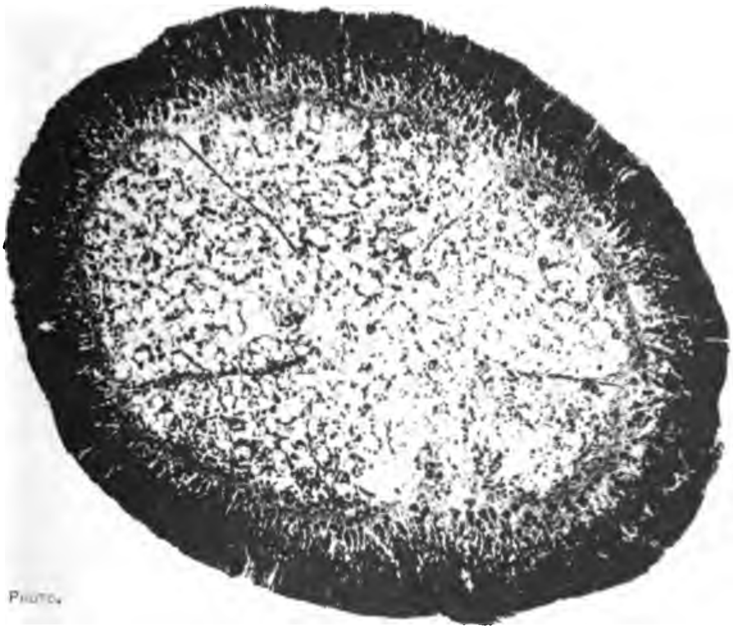

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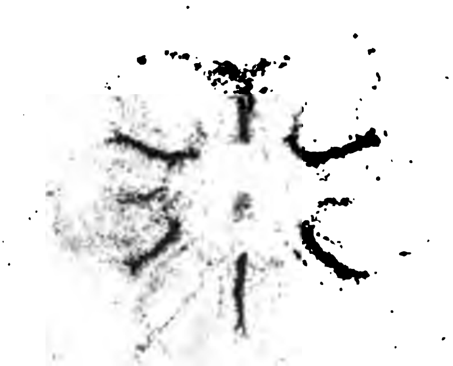

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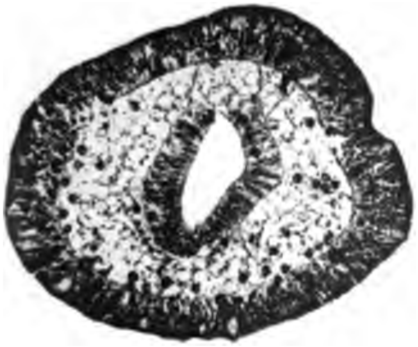

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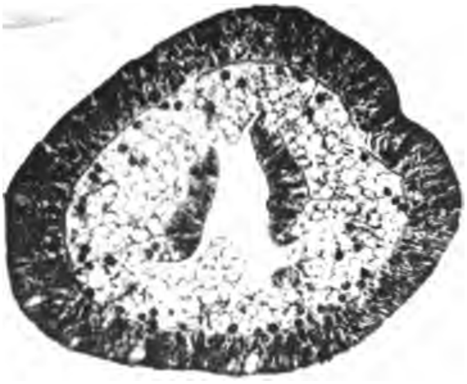

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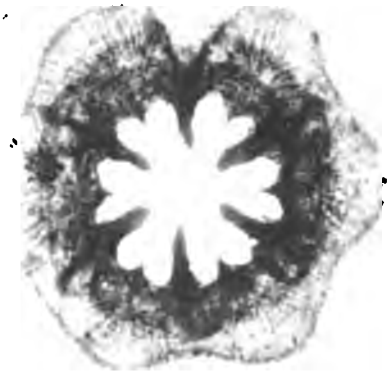

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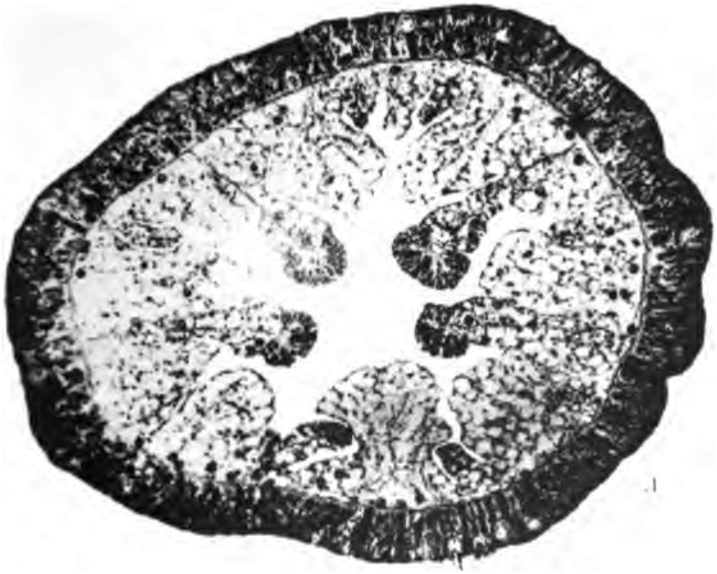

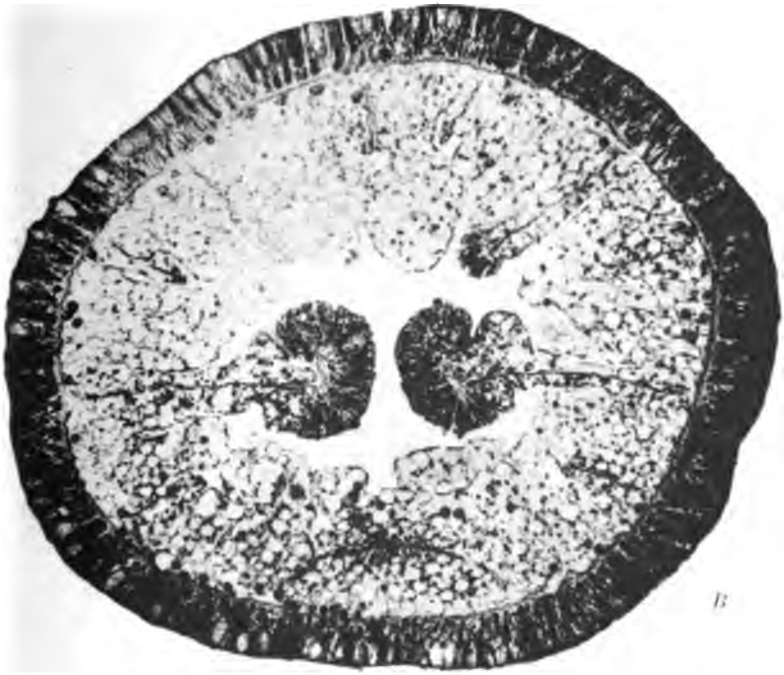

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

VOL. 1.

1. **COLE**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. **GULICK**, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. **BOWDITCH**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. **COE**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. **BIGELOW**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. **MARK**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **BARBOUR**, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. **BLACKMAN**, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. **CONGDON**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. **BLACKMAN**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

VOL. 2.

11. **LINTON**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. **PARKER**, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts and Sci., **43**(16): 413–455. Apr., 1908.
13. **COLE**, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. **MORGULIS**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. **MORGULIS**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. **RAND**, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. **MARK**, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting. *Separate*: 6 pp. Nov., 1909.
18. **SMALLWOOD**, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. **SMALLWOOD**, W. M., AND **ROGERS**, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. **CARPENTER**, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. **ESTERLY**, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. **COWDRY**, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. **GROSS**, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. **SMALLWOOD**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. **Mark, K. L.** — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18): 669–678. April, 1913.
26. **Smallwood, W. M., and Clark, E. G.** — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23** (4): 625–636. Dec., 1912.
27. **Chester, W. M.** — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20): 737–773, 4 pls. May, 1913.
28. **Kutchin, H. L.** — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10): 569–626, 8 pls. Oct., 1913.
29. **Hilton, W. A.** — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1): 113–130. Dec., 1913.
30. **Crozier, W. J.** — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6): 491–492. Febr., 1914.
31. **Parker, G. H.** — The Locomotion of Chiton. 2 p. April, 1914.
32. **Parker, G. H.** — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3): 443–446. April, 1914.
33. **Crozier, W. J.** — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **Crozier, W. J.** — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1): 8–20. Dec., 1914.
35. **Crozier, M. J.** — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577): 28–36. Jan., 1915.
36. **Arey, L. B.** — The Orientation of Amphioxus during Locomotion. Jour. of Exp Zoöl., **19** (1): 37–44. July, 1915.
37. **Crozier, W. J.** — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2): 196–202. Jan., 1915.
38. **Mavor, J. W.** — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9): 183–211, 6 pls. Dec., 1915.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. **Gulick**, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. **Mark**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
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9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
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Vol. 2.

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15. **Morgulis**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. **Rand**, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. **Mark**, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
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20. **Carpenter**, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
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59.11.044:4.36

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

No. 39.

ON CELL PENETRATION BY ACIDS

By W. J. Crozier.

From Science, New Series, Vol. XLII, No. 1090.

CAMBRIDGE, MASS., U. S. A.
November, 1915.

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ON CELL PENETRATION BY ACIDS[1]

Preliminary Note

1. THE water-soluble blue pigment[2] in the cells of a nudibranch, *Chromodoris zebra* Heilprin, is a sufficiently delicate indicator to justify its use for the study of cell penetration by acids. Water extracts of the animal, containing this pigment and other cell materials expressed by grinding, change from a deep blue color with reddish-purple fluorescence to a delicate pink hue at a hydrogen ion concentration of $p_H = 5.6$[3]; the acidity of the body fluids of *Chromodoris* averages $p_H = 7.4$ (27°). The indicator promptly flocculates, in the form of a greenish-blue precipitate, leaving a blue solution, at $p_H = 7.6$. Within the epidermal cells the pigment is also turned green, so that it may be used to measure the penetration of alkalies; it gives results concordant with those obtained with a great variety of tissues by the neutral red method (Harvey[4]), and with neutral red-stained *Chromodoris* cells lacking the blue pigment.

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[1] Contributions from the Bermuda Biological Station for Research, No. 39.

[2] Crozier, W. J., 1914, *Journal of Physiology*, Vol. 47, p. 491.

[3] This point changes somewhat with the age of the extract, in the case of alcohol (95 per cent.) and other permanent solutions of the pigment. The p_H values given were obtained by titration with phosphate and acetate mixtures, checked by gas chain measurements on alcohol and formalin solutions of the pigment.

[4] Harvey, E. N., 1914, Papers from the Tortugas Lab., Vol. VI., p. 133.

The pigment occurs in two forms: as granules scattered through the superficial and deeper tissues, and dissolved in clear globular bodies located within the cells of the outer epithelium, especially along the edges of the mantle and foot. It is totally insoluble in anhydrous acetone, ether, chloroform, xylol and oils. The globules containing it do not stain with fat dyes. I conclude that the pigment is held naturally in water solution.

2. Direct measurements of the speed with which acids penetrate protoplasm were first given by Harvey,[5] who determined the time required for the testis of *Stichopus ananus* to change in color when immersed in 0.01 *N* solutions of a number of acids. I have used pieces of the lateral mantle edge of *Chromodoris* in a similar way, precautions

TABLE I

Penetration of Acids from 0.01 N Solutions

No.	Acid.	Time, Minutes.	
		Chromodoris. Mantle Edge. 27°.0.	*Stichopus ananus, testis* (Harvey).[6]
1	Valeric (Iso-)........	1.9	2–4
2	Salicylic..............	3.5	0.25
3	Formic................	4.5	2–4
4	Hydrochloric........	7.6	9–11
5	Nitric[7]................	8.4	
6	Sulphuric............	8.5	
7	Lactic	8.6	
8	Oxalic..................	8.8	12–15
9	Tartaric...............	13.5	30
10	Citric...................	16.0	40
11	Butyric................	19.0	45–60
12	Acetic..................	75.0	

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[5] Harvey, E. N., 1914, SCIENCE, N. S., Vol. XXXIX., p. 947.

[6] Only those acids which I have studied have been taken from Harvey's table, which includes a number of others.

[7] The differences in penetration-time for Nos. 5–8 are slight at this concentration, but their separation is justified on the basis of the dilution curves.

being taken to insure comparative uniformity of the pieces in the different tests, and find that *at this concentration* (0.01 *N*) the acids employed when arranged in the order of increasing penetration-time form the series shown in Table I. Comparison of this list with that of Harvey discloses that the relative penetrating power of the acids at this concentration is practically identical in the two cases. Some of the differences may be due to the temperatures at which the two sets of experiments were made. The figures for the *Chromodoris* tissue represent the mean of ten concordant experiments at a uniform temperature of 27°.0.

3. Examination of the penetration time of these acids over a *range of concentrations* (0.1 *N* to 0.001 *N*) shows, however, that the series established at the single concentration (0.01 *N*) gives an entirely misleading picture of the penetrating powers of the different acids, which is better judged by the nature of the penetration curves as a whole. According to this view the order arrived at is seen in Table II. The acids studied may be arranged in two groups on the basis of the character of

TABLE II

Penetration Power of Acids

Group I:

Acid	Ionisation Constant (*K*)
Hydrochloric	(100)
Sulphuric	(100)
Oxalic	3.8
Nitric	(100)

Group II:

Formic	0.0214
Salicylic	0.102
Valeric (iso-)	0.0017
Lactic	0.0138
Tartaric	0.1000
Citric	0.0870
Butyric	0.00149
Acetic	0.00180

their penetration-dilution curves.[8] The curves of the second group are all more or less parallel and uniformly concave toward the axis of penetration-time, whereas the curves of acids of the first group (up to 0.002 N) are from the beginning concave toward the axis of dilution. The curves of the two sets cut across one another, as do also some of the curves within each set. The acids of the first set give visible evidence of penetration at higher dilutions ($n/750$) than do those of the second group.

The separation of these two groups of acids is further warranted by the fact that, within certain time-limits, a preliminary exposure of the *Chromodoris* tissue to the action of acids of the second group does not hasten the penetration of acids of the first series, but does that of other acids of the second set.

4. The acids included in my group I. of Table II. are all acids of strong ionization, while those of the other group are of low acid strength. To this extent the rôle of ionization in determining permeability toward acids is made clearer than has hitherto been the case, and it seems probable that these two kinds of penetration curves represent at least two different and distinct methods whereby acids may gain access to the interior of cells. Within each of the two sets of acids the degree of ionization is less important in controlling the speed of penetration. Formic acid occupies a somewhat peculiar position, as do also butyric and valeric; the first substance shows a dilution curve more nearly approaching that of the strong acids, agreeing with its constitution, but the relative positions of butyric and valeric in the series are more likely to be accounted for by their rather high solubility in lipoids.

All the evidence so far available indicates that acids penetrate and combine to various degrees with one or more of several constituents of the cell surface. It is certain, at any rate, that the "lipoid theory" of permeability is not even approximately complete as an explanation. Further attempts to elucidate the significance of the apparently quite general uniformity in the order of cell penetrability for various acids in different animals must await the study of a larger series of substances, especially with reference to the action of acids on penetrability for other acids.[9]

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[8] This series has an important bearing on the interpretation of sensory stimulation by acids, a matter which first turned my attention to this problem.

W. J. CROZIER

AGAR'S ISLAND, BERMUDA

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[9] Details, covering additional points not here considered, will be found in a paper to appear in the *Journal of Biological Chemistry.*

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2. **Gulick**, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
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6. **Mark**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
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Vol. 2.

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59.11.853:6

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No. 40.

REGARDING THE EXISTENCE OF THE 'COMMON CHEMICAL SENSE' IN VERTEBRATES.

By W. J. Crozier.

From The Journal of Comparative Neurology, Vol. XXVI, No. 1.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 268.

REGARDING THE EXISTENCE OF THE 'COMMON CHEMICAL SENSE' IN VERTEBRATES[1]

W. J. CROZIER

I. In a recent paper by Coghill ('14) exception is taken to the view held by Herrick ('08), Sheldon ('09), Cole ('10), and Parker ('12), that there is in vertebrates a set of receptors (free nerve terminations) which are responsible for reactions to rather high concentrations of chemicals when applied to moist peripheral surfaces. The theoretical significance attached to this 'common chemical sense' (Parker, '12) makes it appropriate to bring forward certain facts which may to some extent serve to clear up the situation.

The evidence adduced by Coghill is this: that in Amblystoma larvae, before the establishment of the definitive nervous system, tactile and chemical irritability appear concomitantly and, so far as studied, remain completely parallel in development; and that if larvae be placed in irritating solutions of hydrochloric acid (as dilute as $\frac{N}{10,000}$), the epithelial cells become visibly disrupted, the reaction of the larvae being correlated with the disintegration of the skin and a gradual disappearance of reaction to tactile stimuli.

His conclusion, that there is not present in the skin any normal irritability to acid, is probably correct for the larvae experimented upon; but it by no means follows that the same conditions obtain in the adult. Coghill argues, however, that the permanently embryonic cells of the germinative layer in the skin of amphibians and fishes when exposed to the high concentrations of acid ($\frac{N}{40}$) used by Parker and Sheldon act as do the superficial ciliated cells of the Amblystoma larval skin. Because they are bound down by a "thick, less sensitive, and more impervious layer of cells," the disintegration of the sensitive cells would cause exceedingly violent disturbances, presumably affecting tactile and pain terminals; and further, that it has not been satisfactorily demonstrated that it is possible (as claimed by Sheldon and by Cole) to effect a separation of tactile and 'common chemical' irritability in fishes and amphibians.

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[1] Contributions from the Bermuda Biological Station for Research. No. 40.

This general argument is somewhat strengthened by the fact (to which Coghill does not refer) that aquatic vertebrates possessing a soft slimy skin—cyclostomes, eels, catfish, Necturus—are known to react to local irritation by chloroform and other substances by the expulsion of mucus and even of entire gland cells. The reaction time of this response is not known accurately, though it is judged to be much greater than that, for example, of the catfish as a whole when stimulated by hydrochloric acid applied to its trunk region; still, it is not inconceivable that mechanical deformations brought about in this way might provide stimuli for tactile nerve endings, provided the mucus response were sufficiently sharp and prompt.

II. The exact manner in which solutions are to produce the hypothecated effects upon the cells of the germinative layer is left entirely untouched by Coghill. The rapidity of the responses given by the catfish and Necturus immediately negatives the idea that osmotic transfer of water is the agency of stimulation. In the case of the spinal frog, as studied by Braeuning ('04), Loeb ('05), and Cole ('10), though the reaction times are rather long, there is abundant evidence that other than osmotic factors are at work. The most important point which arises for consideration is the extent to which chemical stimulants actually penetrate the skin, i.e., the degree to which the cells of the germinative layer are exposed to the action of the stimulant. In addition to the rapidity of the reactions under discussion, it is to be remembered that they are excited by acids, salts, alcohols, and a variety of other substances.

That the skin of aquatic animals is not to any appreciable degree damaged by the agents responsible for 'common-chemical-sense' reactions, is clearly indicated by such facts as the following:

1. High concentrations of acids ($\frac{N}{10}$), salts (2N), alkalies ($\frac{N}{10}$) and alkaloids ($\frac{M}{100}$), which evoke prompt and vigorous reactions from the earthworm, Eisenia foetida, cease to cause any disturbance the moment the stimulant is washed off by immersing the animal in water. If any serious disintegration were produced by these solutions, it would be reasonable to expect the continuance of activity after the external supply of the stimulant had been removed.[2]

2. Holothurians, such as H. surinamensis (Crozier, '15 c) and Stichopus moebii (Crozier, '15 b), possess skin pigments whose loss is a fairly good indicator of changes in permeability. Yet in no case on the application, by the pipette method, of the stimuli used by Parker ('12) was there any indication of pigment loss accompanying reaction.[3] This is true also of the nudibranch, Chromodoris zebra.

III. The value of the intracellular indicator of Chromodoris in the study of cell penetration by acids has been pointed out elsewhere (Crozier, '15 b). When small volumes of acids, even in $\frac{N}{10}$ solution, are used to stimulate Chromodoris, there is no visible evidence that they penetrate the skin. In fact the time required for the penetration of the acids is quite high (see also Harvey, '14), the most rapid rate observed being with $\frac{N}{50}$ iso-valeric acid, where penetration requires 1.5 minutes.

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[2] Experiments with earthworms also disclose certain important deficiencies in the method whereby the animals to be tested are entirely immersed in the stimulating solution. If the reactions are to be studied quantitatively, there are features undoubtedly obscured by this procedure. For example, the time elapsing between the complete immersion of an earthworm (Eisenia foetida) in a $\frac{N}{10}$ lithium chloride solution and the instant the first writhing movement appeared, was found to be at least ten times greater than the reaction time of the same worm when part of it (the anterior end) was stimulated. The speed of reaction is in part conditioned by the number of receptors affected, but this does not analyze the situation completely. It is quite probable that the responses observed by Coghill are not at all due directly to sensory stimulation by acid.

[3] I have observed that under certain circumstances Ptychodera sp.—a Bermudan enteropneust—reacts to chemical irritation by extruding a yellowish pigment. There is ground, however, for believing that this is an instance comparable to that of Lumbriconereis (Kschischkowski, '12), in which K salts have apparently a more or less specific action in producing the response.

For $\frac{N}{10}$ HNO_3 the penetration time found (Chromodoris tissue) was 3.5 minutes; for $\frac{N}{20}$, 4.3 minutes; whereas Necturus, under water, was found to react to $\frac{N}{10}$ HNO_3 (0.5 cc. of the solution being applied with a pipette) in 1.5 seconds when stimulated on the dorsal surface of the head, 5 seconds on the lateral mid-body surface, and 2 seconds on the tail; with $\frac{N}{20}$ HNO_3 the reaction times for corresponding locations were 5, 10, and 6 seconds respectively.[4] Even here, though the pipette tip was held within approximately a centimeter of the animal's surface, the concentration of the solution in the pipette does not represent the concentration which actually reaches the stimulated surface, as already noted by Parker ('12, p. 222). The conclusion must therefore be, that acids do not penetrate the skin with sufficient rapidity to affect the cells of the germinative layer. This conclusion must also be extended to alkalies, since Harvey ('10) and others have shown the high impermeability of cells to strong hydroxides.

IV. In the light of the evidence just discussed, it seems improbable that the high concentrations of irritants employed by Parker and others in studying reactions attributed to the common chemical sense produce any violently disruptive effects when applied to the skin of aquatic animals. Indeed, so far as concerns the skin as a whole, they do not penetrate at all,[5] and the cells of the germinative layer cannot be held to be exposed to their action.

This conclusion was verified, in the case of the spinal frog, by experiments of the following type:

1. The reaction time for the withdrawal of the frog's foot from $\frac{N}{10}$ $CuSO_4$, is about 7 seconds (at 24°.9). After being withdrawn from the solution, the feet continue for some time to be spasmodically contracted. These subsequent contractions are entirely inhibited the instant a foot, just retracted from $CuSO_4$,

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[4] The reaction times were measured at 20°, while the penetration of the acid was studied at 27°; the speed of penetration decreases markedly with falling temperature.

[5] The mode of action of the stimulating agent on the individual receptor is entirely another question.

is dipped into a weak solution of $K_4Fe(CN)_6$, which precipitates the copper held by the mucus of the foot. A similar result was obtained with copper acetate. Washing the foot with distilled water does not lead to a cessation of the contractions, because, after exposure of the foot to certain solutions (Loeb, '05), water stimulates.

2. After two to four stimulations of the frog's foot by relatively strong solutions of either copper acetate or ferric sulphate, the stimulated area was sectioned and tested microchemically for the penetration of the copper or iron. The metals were found in the mucus of the surface of the foot, and in several instances doubtful traces were discovered between cells of the extreme outer portion of the epidermis. No evidence was found of any disruption of the germinative layer.

3. As a stimulating agent whose penetration would readily be visible, the action of picric acid was studied. The outcome of experiments with this substance at several concentrations may be illustrated by the records here copied:

Experiments H and L. Picric acid, $\frac{M}{50}$. R. T. = Reaction time in seconds. Successive tests at 5 min. intervals. N. R. = No reaction.

NO.	R.T.		NOTES
	H	L	
1	7.8	2.1	No staining.
2	12.8	6.6	Slight staining.
3	16.8	7.8	Staining progressively brighter.
4	30.2	11.8	
5	40.0*	24.6	
6	45.0	43.2*	
7	N.R.	N.R.	No reaction to $\frac{N}{5}$ formic acid.

* Not reactive to pinching beyond this point.

From these and similar tests, it was concluded that the penetration of the stimulant rapidily renders the frog's foot less reactive by killing the superficial cells. In the case of picric acid it is probably the H ion which is mainly concerned in stimulation, since $\frac{M}{50}$ ammonium picrate is entirely ineffective, neither does it easily stain the frog's foot. This does not, however, signify that other acids behave as does picric, since as many as 20 successive reactions may be obtained from $\frac{N}{10}$ HCl. Moreover, the staining is not directly correlated with the stimulating effect, since the skin of the frog's foot was stained by immersion for 3 minutes in $\frac{M}{200}$ picric acid without producing any reaction. The skin of Necturus and the catfish may be stimulated with $\frac{M}{50}$ picric acid without producing any visible stain.

V. I have repeated on the frog's foot the experiment of Sheldon ('09) and Cole ('10) regarding the separation of tactile irritability and responsiveness to irritating solution, by treatment with cocaine. A 0.5 per cent solution of cocaine hydrochloride was used, and the tests were made by comparing at intervals the reactions of the cocainized foot with those of the untreated one. After about 20 minutes' immersion, the reaction time of the cocainized leg to $\frac{N}{10}$ formic acid (chosen as a powerful stimulant) was usually twice that of the normal foot; after about an hour, varying in some tests to an hour and a half, the cocainized foot no longer reacted to pinching, but gave good responses to the acid with reaction times of 10–15 seconds, still about twice the reaction time of the non-narcotized leg.

It is therefore possible, I believe, to effect experimentally a separation of sensitivity to mechanical and to chemical stimulation in the frog's foot.

There can be no question of the distinctness of the human sensation attributed to the common chemical sense (Parker, '12; Parker and Stabler, '13) as compared with any tactile sensation; and from tests made upon cocainized areas of my own mouth,[6] I am certain that the two sets of receptors are not only qualitatively distinct as regards the sensations with which they are connected, but also may be separated by the use of cocaine.

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[6] These tests concerned mostly the inner surface of the cheek.

SUMMARY

1. There is positive evidence, in the case of alkalies, acids, and certain salts, that solutions supplying stimuli for the common chemical sense do not penetrate the skin of aquatic animals, nor when applied from a pipette do they damage the skin to any extent.

2. There is consequently no ground for Coghill's assumption that the cells of the germinative layer of the epithelium of fishes and amphibians are exposed to the action of the stimulating agent and thereby disrupted; and there is no histological evidence of disruption.

3. The reactions attributed to the common chemical sense depend upon a group of sense organs distinct from those sensitive to mechanical stimulation.

REFERENCES

BRAEUNING, H. 1904 Zur Kenntnis der Wirkung chemischen Reize. Arch. ges. Physiol., Bd. 102, p. 163–184.

COGHILL, G. E. 1914 Correlated anatomical and physiological studies of the growth of the nervous system of Amphibia. I. The afferent system of the trunk of Amblystoma. Jour. Comp. Neur., vol. 24, p. 161–233.

COLE, L. W. 1910 Reactions of frogs to chlorides of ammonium, potassium, sodium, and lithium. Jour. Comp. Neur., vol. 19, p. 273–311.

CROZIER, W. J. 1915 a The behavior of an enteropneust. Science, N. S., vol. 41, p. 471–472.
(Note—Lines 5 and 6 [p. 472] have been accidentally interchanged by the printer.)

1915 b The rhythmic pulsation of the cloaca of holothurians. Ibid., p. 474.

1915 c The sensory reactions of Holothuria surinamensis. Zool. Jahrb., Abt. Physiol. [Not yet published].

1915 d On cell penetration by acids. Science, N. S., vol. 42, p. 735–736.

HARVEY, E. N. 1910 Studies on the permeability of cells. Jour. Exp. Zoöl., vol. 10, p. 507–556.

1914 Cell permeability for acids. Science, N. S., vol. 39, p. 947–949.

HERRICK, C. J. 1908 On the phylogenetic differentiation of the organs of taste and smell. Jour. Comp. Neur., vol. 28, p. 157–166.

KSCHISCHOWSKI, K. 1912 Neue Beiträge zur Pigmentabsonderung bei Anneliden. Zentralbl. f. Physiol., Bd. 26, p. 528–532.

LOEB, J. 1905 Production of hypersensitivity of the skin by electrolytes. In: Studies in General Physiology, pt. II, Chicago. pp. 748–765.

PARKER, G. H. 1912 The relation of smell, taste, and the common chemical sense in vertebrates. Jour. Acad. Nat. Sci. Phila., Ser. 2, vol. 15, p. 221–234.

PARKER, G. H., AND STABLER, E. M. 1913 On certain distinctions between taste and smell. Amer. Jour. Physiol., vol. 32, p. 230–240.

SHELDON, R. E. 1909 The reactions of the dogfish to chemical stimuli. Jour. Comp. Neur., vol. 19, p. 273–311.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. **Gulick**, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. **Mark**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **Barbour**, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. **Linton**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. **Parker**, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. **Cole**, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. **Morgulis**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. **Morgulis**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. **Rand**, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. **Mark**, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting. *Separate*: 6 pp. Nov., 1909.
18. **Smallwood**, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. **Smallwood**, W. M., **and Rogers**, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. **Carpenter**, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. **Esterly**, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. **Cowdry**, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. **Gross**, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. **Smallwood**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. **Mark**, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. **Smallwood**, W. M., **and Clark**, E. G. — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. **Chester**, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773, 4 pls. May, 1913.
28. **Kutchin**, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569–626, 8 pls. Oct., 1913.
29. **Hilton**, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. **Crozier**, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. **Parker**, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. **Parker**, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. **Crozier**, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **Crozier**, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. **Crozier**, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. **Arey**, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. **Crozier**, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. **Mavor**, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. **Crozier**, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. **Crozier**, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
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3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
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24. **Smallwood**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

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28. **KUTCHIN, H. L.** — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49**(10) : 569–626, 8 pls. Oct., 1913.
29. **HILTON, W. A.** — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. **CROZIER, W. J.** — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. **PARKER, G. H.** — The Locomotion of Chiton. 2 p. April, 1914.
32. **PARKER, G. H.** — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16**(3) : 443–445. April, 1914.
33. **CROZIER, W. J.** — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **CROZIER, W. J.** — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. **CROZIER, W. J.** — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. **AREY, L. B.** — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. **CROZIER, W. J.** — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. **MAVOR, J. W.** — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. **CROZIER, W. J.** — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. **CROZIER, W. J.** — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.

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BY W. J. CROZIER.

Contributions from the Zoological Laboratory of the Museum of Comparative Zoology at Harvard College, No. 270, and the Bermuda Biological Station for Research, No. 41.

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CONTENTS.

Introduction.... 255
The indicator.... 257
Method.... 259
Penetration speed and concentration.... 264
Comparisons with stimulation.... 270
Summary.... 276
References.... 278

INTRODUCTION.

A study of the chemoreceptors of the earthworm *Eisenia foetida*, begun at the suggestion of Professor G. H. Parker, resulted in the accumulation of data which could not be analyzed in terms of current ideas relating to cell permeability and its rôle in stimulation. At the same time it led to certain views regarding the problem of irritability; namely, that this question may best be studied with the sense organs of animals of moderate structural complexity, rather than in simple[1] protozoa or in relatively non-specialized cells. In a ceptor cell the processes of irritability stand forth with especial clearness, because less obscured by the course of other activities than is probably the case with the majority of tissue cells; irritability is so predominantly the function of the ceptor that it may here be studied in a reasonably pure condition.[2] Organs of chemical sense seem particularly well adapted for such experiments. Moderate morphological complication is a necessary restriction for technical reasons, since it is only with animals of not too high an organization that quantitative methods of experiment involving the measurement of responses may successfully be used.

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[1] Dobell (1914, page 181) states: "It is safe to prophesy that when the known facts have been doubled or trebled, the ironical statement . . . . that 'the Protozoa are the simplest organisms in which to study the great problems of biology' will disappear from biological literature."

Part of the study referred to dealt with stimulation by acids, and the facts observed in this connection were responsible for the present work on the penetration of cells by acids.

Previous to the recent paper of Harvey (1914), cell penetrability for acids had not been studied by any direct and approximately quantitative method, largely because, as Harvey remarks, cells containing indicators appropriate for this work have seldom been observed.[3] He was able to utilize the pigmented gonidial filaments of a holothurian, *Stichopus ananus* Jaeger, and measured the time required for the testis of this animal to change color when placed in 0.01 N solutions of a variety of acids. The results of his experiments with acids of this concentration led Harvey to the view that probably lipoid solubility, or capillary activity, and affinity for proteins of the cell surface were both factors in determining the particular penetration speed observed with a given acid, but that there was no agreement between penetration speed and ionization.

During the summer of 1913 I found that the blue pigment of a nudibranch, *Chromodoris zebra* Heilprin, gave an extract with dependable qualities as an indicator, and in a brief note (Crozier, 1914) I described some properties of the substance. It was also found that acids would change the color of the indicator within the cell.

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[2] I have since observed that a similar idea has been developed by Lucas (1909, particularly page 328): "The physiology of the cell must begin, not with that maid-of-all-work, the unicellular organism, but with such cells as are as nearly as possible unifunctional." "The only practical method for the study of any function will be to investigate that function first in some cell in which it appears in a highly elaborated state."

[3] The statement is often made that indicators are of rare occurrence in animals, but I have discovered such materials during the last 3 years in such widely separated forms as: a balanoglossid, a common Bermudian and West Indian tunicate, and an annelid, in addition to the one herein described.

Harvey's list of acids, though embracing a number of substances, was studied at only one concentration, and previous work on stimulation by acids had convinced me that series purporting to indicate the relative physiological activity of acids were not likely to be of general value when constructed on such a basis. This expectation has been completely justified (Crozier, 1915). It is with this problem, the penetration rate of acids as a function of concentration, and the interpretation of stimulation by acids, that the present account is concerned. The conclusions to which I have come regarding permeability to acids are in a way similar to those of Harvey, aside from the part assigned to ionization, but the method of analysis is different and based upon more complete data.[4]

The Indicator.

The only characteristics of the *Chromodoris* indicator which are important here are (a) its mode of occurrence and (b) the influence of various conditions upon the magnitude and rapidity of its color changes. Some hints have been obtained as to the chemical nature of the material and its significance for the so called warning coloration of the animal, but these will be described in another place.

For example, this indicator gives positive results with nine tests for alkaloids, but otherwise gives no reaction with protein precipitants; it appears to be intimately connected with the animal's spicy odor and general immunity from enemies. The pigment appears to bear some relation to the blue material described by von Zeynek (1901, 1902) from the fish *Crenilabrus*. From the fact that the blue substance is decolorized on reduction and may be restored to its original hue by H_2O_2, some writers would doubtless regard it as a respiratory pigment.

(*a*) *Occurrence.*—The position of the pigment in the cell cannot be made out in sections, because it dissolves. Observations are therefore restricted to teased cells and tissues manipulated in a compressorium. Such observation shows that several forms of the pigment are present. Along the border of the mantle it can be seen that by far most of the material is situated at the base of the epithelial cells, in the form of sac-like bodies of variable size and (apparently changeable) shape. But blue granules occur quite near to the outer surface, and the small spherical bodies which occur in the mucus on the animal's surface are strongly tinged with blue.

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[4] The control of permeability to acids by salts, and other phases of this problem, are also being studied.

The coloration of *Chromodoris* depends upon the blue pigment and a yellow one. Along the border line between blue and yellow patches, cells can be made out which contain both yellow and blue pigment, like the doubly pigmented erythrophores of *Ostracion* described by Ballowitz (1913); but such cells are infrequent.

(*b*) *Color Changes.*—The yellow pigment is not soluble in water and cannot be extracted by aqueous alcohol, aqueous acetone, or pyridine. It is soluble in xylol, full strength alcohol, acetone, and other fat solvents. Its globules stain with dyes that color fat.

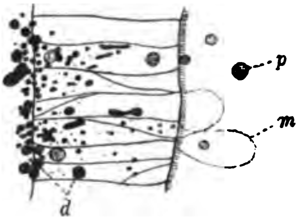

Fig. 1. From a camera sketch of a compressed preparation of the edge of the mantle. *d* = dense blue bodies; *p* = pale spherical masses; *m* = mucus. × 125.

The blue bodies indicated below the basement membrane were located in near-by cells of the epithelium, the outlines of which are not shown at this focus.

The blue substance, which is soluble in water, is the only indicator involved. Pyridine, a weak base, apparently extracts it in the least changed condition, the solution being blue or blue-green. Alcoholic or aqueous extracts are at first blue with a reddish fluorescence, but subsequently turn purple, with a strong blue fluorescence. As an indicator this pigment behaves somewhat like litmus, though its tints are more pronounced. Solutions of the pigment in fresh water or in 4 per cent solutions of formalin in either fresh water or sea water, and alcoholic extracts or aqueous solutions of the residue from an evaporated pyridine extract, give sensibly the same end-point when a few drops are added to 10 cc. of test solutions. The hydrogen-ion concentration for the transition blue → pink, determined by phosphate and acetate mixtures, is the same, $p_H = 5.6 \pm$; this holds for solutions containing sea-water salts ($NaCl + KCl + CaCl_2$, at $\frac{5}{8}$ M total concentration), therefore corresponding presumably to the condition in the cell. 0.001 N solutions *in vitro* of the eighteen acids I have used effect the blue → pink transformation instantly. Dilute solutions containing the proteins of *Chromodoris* may give apparent end-points as low as $p_H = 4.7$.[5]

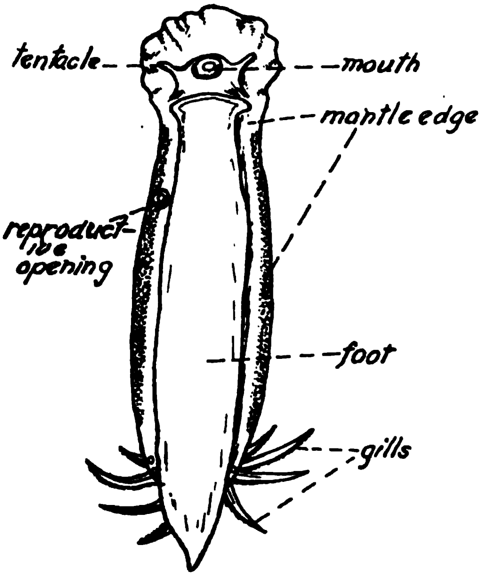

FIG. 2. *Chromodoris zebra*, diagrammatic ventral view; stippled areas show regions from which pieces were cut for the study of acid penetration. × ¾.

Method.

Pieces were cut from the lateral mantle edge of *Chromodoris* shown by Fig. 2. The animals were in every case freshly collected and in good condition, though I cannot agree with Smallwood (1910, page 140) that these animals do not well survive laboratory confinement. The pieces excised were about 10 mm.

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[5] Compare Harvey (1913).

long by 5 to 6 mm. broad at the widest place, but tapering to a point at either end, and usually 2 to 3 mm. thick along the line of cutting, thinning rapidly to less than 0.5 mm. at the extreme outer edge. The penetrability of the different regions of an animal varies considerably (Table I), and for animals of different sizes the speed with which acids produce color change in pieces cut from corresponding locations is somewhat related to the length of the individual, as indicated in Table II, smaller speci-

TABLE I.

Comparative Penetrability of Different Parts of Chromodoris to 0.01 N Acetic Acid. Time of Visible Penetration in Minutes.

	Penetration.
	min.
Rhinophores	1.0
Tentacles	1.5
Buccal mantle	4.0
Gills	4.0
Ventral surface of marginal mantle	75
Dorsal " " " "	>120
Foot, edge	100
Buccal margin of pharynx	35
Inner wall " "	90

TABLE II.

Penetration of Pieces Cut from the Midlateral Mantle of Individuals (Chromodoris) of Different Sizes. The Pieces Were All of the Same Size, Two or Three from Each Individual. 0.1 N HCl.

Animal.		Penetration. (27°C.)		
No.	Length.			
	cm.	*min.*	*min.*	*min.*
1	2.5	1.00	1.25	1.00
2	3.7	1.75	1.50	
3	3.9	1.75	2.00	2.25
4	5.5	1.00	1.25	1.20
5	6.2	3.00	3.25	
6	6.4	3.50	3.00	3.25
7	7.8	3.75	4.00	
8	10.4	2.75	3.50	3.25

mens tending to show greater penetrability. Consistent results were, however, easily obtained when these size and location factors were made uniform, and especially when the under surface of the mantle was employed.[6] Animals 8.5 to 10 cm. long were used.

The density of the blue coloration varies widely in different individuals, though the general scheme (Table I) is consistent for any one animal, as far as the area we are considering is concerned (Fig. 2). Material was therefore selected from animals showing a smooth blue pigmentation of the under surface of the mantle.

The source of error involved at this point lies less in the different metabolic conditions with which the several degrees of pigmentation may be correlated, and in the fact that the concentration of the indicator may affect its rate of color change by acids, than in one's inability to judge accurately as to the exact point at which the pink hue becomes evident. *Chromodoris* shows four rather well defined conditions as regards the character of the blue mantle pigmentation; these may be termed the albino (see Smallwood, 1910, page 141), the blue, the purplish blue, and the spotted. The spotted condition is one in which the blue coloration is confined to circular dots less than 1 mm. in diameter, which are separated by distances usually of several mm., the intervening surface being pure white or slightly tinged with blue. These color varieties are not very clear-cut, the blue showing occasionally a spotting of denser color, but for convenience I have used them in Table III to show the variation in penetration time observed with the different modes of coloration.

TABLE III.

Penetration of Pieces of the Lateral Mantle by 0.01 N HCl (27°C.).

Coloration.		Penetration.				
		1	2	3	4	5
		min.	*min.*	*min.*	*min.*	*min.*
A	Purplish blue....	5.5	5.0	6.7	6.9	7.3
B	Blue.............	8.0	6.9	7.2	9.0	5.4
C	Spotted..........	8.9	9.6	7.5	8.1	7.3

Chromodoris is an hermaphrodite, and animals of the size we are dealing with reproduce at all times of the year; therefore sex the effect of heat upon the indicator within the cell is to be ascribed to the increased alkalinity brought about by the thermal coagulation of proteins (Quagliariello, 1912). Cells killed by formalin do not give constant results, but the indication (Table IV) is (1) that cells so killed are more easily penetrated than those not previously killed, and (2) that the increased penetrability is not such as to make the cells readily permeable for all acids, though it amounts to about twice that of cells treated with acid while living.

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[6] Part of the pigment on the dorsal surface seems to be in a different physical or chemical condition, such that it is not easily dissolved out or changed in color.

Penetration Speed and Concentration.

Proceeding in the manner indicated by the previous section, the time of penetrations from a number of concentrations was determined for eighteen acids. The results are summarized in Table VII. Ten or more observations were made at each dilution. The general concordance of the separate measurements, which naturally is better at higher concentrations, may be illustrated by the records in Tables V and VI.

As I have elsewhere remarked (Crozier, 1915), the series of penetration rates from 0.01 N solutions shows a noteworthy correspondence with Harvey's (1914) measurements at the same dilution. The reasonable closeness of this agreement (Table VIII), considering that two widely separated animals were employed, and that in one case a portion of the integument and in the other an internal protected organ (testis) furnished the cells experimented upon, makes it logical to assume that a similar order of acid penetration is generally valid.

TABLE V.

*Penetration of Chromodoris Tissue by 0.01 N HCl (Experiment 10.13).**

No.	Penetration.	No.	Penetration.
	min.		*min.*
1	9.0	6	8.0
2	7.0	7	8.25
3	8.0	8	6.9
4	7.5	9	7.25
5	5.5	10	8.5

Mean.. 7.6±0.78

* A second series, 3 months later, gave 7.5±0.50.

TABLE VI.

Experiments with Oxalic, Monochloroacetic, and Acetic Acids.

No.	Penetration.		
	Oxalic, 0.002 N.	Monochloroacetic, 0.005 N.	Acetic, 0.01 N.
	min.	*min.*	*min.*
1	18.0	18.3	57
2	19.5	17.0	90
3	20.6	13.0	70
4	17.5	14.2	67
5	19.6	17.5	85
6	16.3	12.5	60
7	19.5	16.5	86
8	18.0	20.0	78
9	21.0	11.3	80
10	25.0	16.0	76
Mean	19.5 ±1.6	15.6 ±2.4	75 ±9.1

TABLE VII.

Acid.	Penetration time in minutes (27°C.).																		
Normal dilution.	10	15	20	50	75	100	125	150	200	250	300	325	350	400	500	650	700	750	1,000
Hydrochloric	2.9			5.8	7.0	7.6				9.5					12.5			16.3	20.0
Sulfuric	2.7		3.2	5.9	7.5	8.5		10.5			13.5				17.0			24.7	
Oxalic	4.0			6.0	8.0	8.8				13.5					19.5	25.0			
Nitric	3.6			5.8		8.4				14.5			17.7		22.0		30.0		
Formic	1.75				4.0	4.5		9.2	13.0		15.0				24.5				
Salicylic				1.8	2.1	3.6	6.4	8.0	11.6	16.3	18.5	32.0			56.5				
Valeric (iso-)				1.2	1.5	1.9		6.0	12.0		26.0			55.0	70.0				
Monochloroacetic	5.6			7.4		10.0			15.5		24.0								
Malonic			4.7			10.0		15.2	22.5	24.0	36.0								
Benzoic						9.7		14.8		29.7									
Lactic	4.6			6.0	7.9	8.6	11.5		21.3	32.5									
Tartaric	5.5			8.8		13.5			32.0	60.0									
Malic	5.9		6.1			14.5			37.5	67.0									
Citric	7.0					16.0		26.0	40.0										
Succinic		6.9				16.5			45.0										
Butyric	7.6			12.6	15.0	19.0		30.0	50.0										
Propionic	7.1					30.0		55.0											
Acetic	11.9			19.5	30.5	75.0													

TABLE VIII.

Cell Penetration from 0.01 N Solutions.

Acid.	*Chromodoris*, mantle edge (27°).	*Stichopus*, testis (Harvey).
	min.	*min.*
Valeric (iso-)	1.9	2–4
Salicylic*	3.6	0.25
Formic	4.5	2–4
Hydrochloric	7.6	9–11
Nitric	8.4	
Sulfuric	8.5	
Lactic	8.6	
Oxalic	8.8	12–15
Benzoic*	9.7	0.25
Monochloroacetic*	10.0	2–4
Malonic	10.0	30
Tartaric	13.5	
Malic	14.5	40
Citric	16.0	
Succinic	16.5	—
Butyric	19.0	45–60
Propionic	30.0	
Acetic	75.0	

* The acids not corresponding in the two series are marked with an asterisk.

The curves of Fig. 3 show the relation between normality dilution (*i.e.*, liters containing one equivalent) and average penetration rate. They bring out features of the penetration process which measurements at a single dilution (Harvey, 1914) leave obscure. Series showing the relative penetrating power of acids vary with the concentration chosen (Crozier, 1915) and it follows that existing correlations of the mode of penetration for different acids with lipoid solubility, toxicity, and the like, must be revised. The form of the dilution curve as a whole must be used to determine the position of an acid in the penetration series.[8] On this basis the acids studied may be separated into two groups, an end also attained, though with less accuracy, by consideration of the limiting concentrations beyond which no color change is effected inside the cell.

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[8] A consideration of this kind applies likewise to studies on the effects of acid solutions of equal hydrogen-ion content.

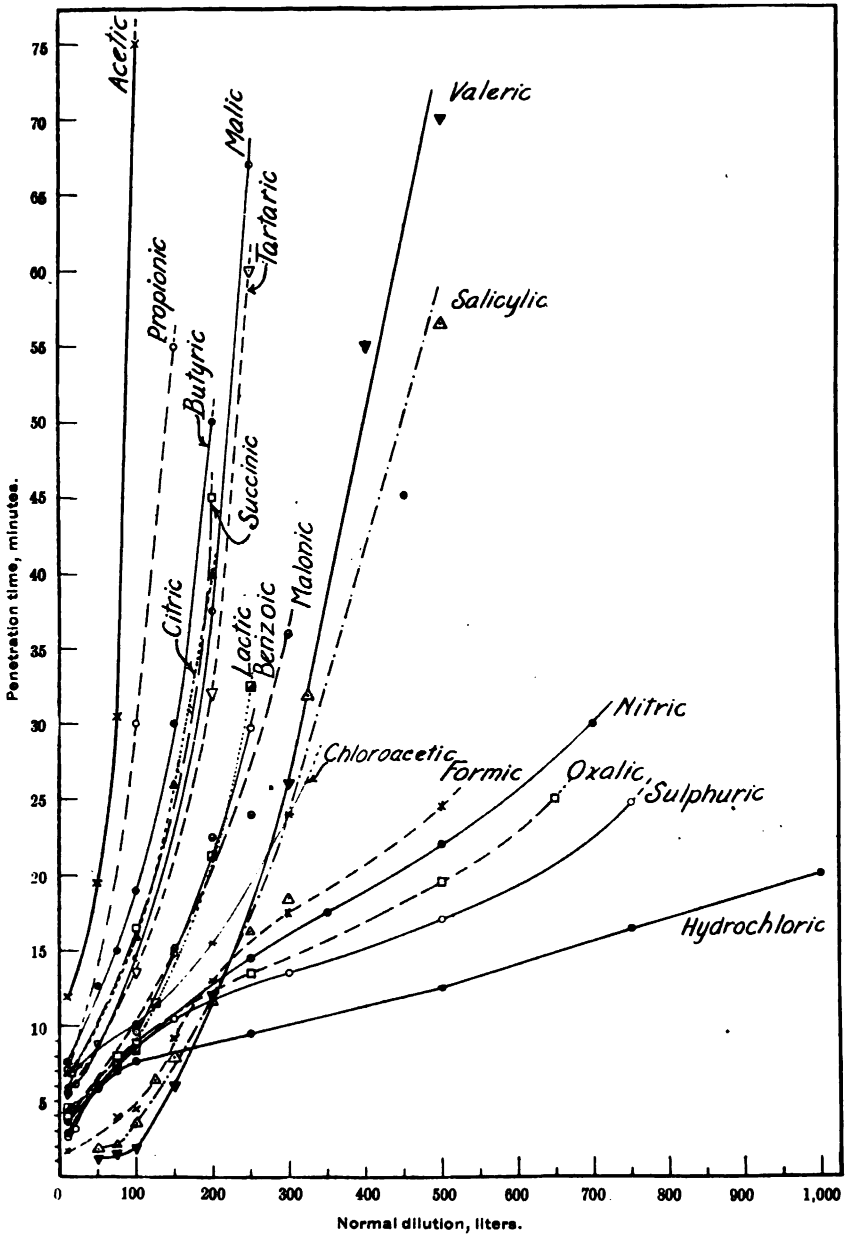

Fig. 3. The relation between dilution and time of penetration.

Outward diffusion of pigment occurs in acid solutions but this diffusion does not bear any constant relation to the time occupied by the internal color change, being variable and depending in part upon the degree to which the piece of tissue is contracted or passively stretched. The use of this outflow of pigment as an index of permeability changes is therefore not free from an objection which may be urged against much recent work on quantitative aspects of the permeability problem; namely, that cell pigment may be squeezed out in purely mechanical fashion, or that mechanical stresses may control the speed and extent of pigment loss. In general, with solutions giving rapid penetration, the spontaneous outward movement of pigment occurred after the color change within the cell; with lower concentrations, before an internal condition of acidity had been made visible. Moreover, as will be shown subsequently, it is possible experimentally to increase or decrease the penetration time of a given acid solution, and in certain of these cases the state of permeability as revealed by pigment diffusion is exactly the reverse of that shown by the speed of acid penetration.

The order of decreasing efficiency in penetration for the eighteen acids studied is approximately as indicated in Table IX, where position in the series is compared with ionization strength.[9]

At first sight there is little precise concordance between penetration speed and ionization, but from the tangle of curves the following sets of chemically related acids may be taken as probably significant.

1. The acids of high ionization are obviously set apart from the others by the character of their dilution curves, while as a whole the most weakly ionized acids are at the other end of the series; and I take the relative position of the members of these two groups to indicate that ionization is the primary determinant of cell penetrating power, but that some other factor[10] controlling entrance into the cell is also operative. On the one hand the ionization of HCl, H_2SO_4, $(COOH)_2$, and HNO_3[11] is so great that the second factor has relatively free sway in fixing their individual position, while, on the other hand, the ionization of the fatty acids is so weak that here also another factor determines the order of penetration, excepting that of formic acid, which is ioni-

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[9] For the first $H^{\cdot}$.

[10] It is hardly probable, and certainly not necessary, that this second factor should be the same for different acids.

[11] Borowikow (1913) noted the order $HCl > H_2SO_4 > HNO_3 > (COOH)_2$ for the acceleration of plant growth.

cally stronger than the others (compare its different type of curve).

2. Among the weak fatty acids the order of penetration is: *valeric* > *butyric* > *propionic* > *acetic*; some clue to the mechanism of the process is clearly afforded by the fact that this is precisely the order of their action upon the surface tension of water and of their solubility in xylol (Harvey, 1914, page 948).[12]

TABLE IX.

Order of Penetration Compared with Ionization.

Acid.	Ionization constant.
Hydrochloric	(100.0)
Sulfuric	(100.0)
Oxalic	3.8
Nitric	(100.0)
Formic	0.0214
Salicylic	0.1020
Valeric (iso-)	0.0017
Monochloroacetic	0.155
Malonic	0.163
Benzoic	0.0060
Lactic	0.0138
Tartaric	0.100
Malic	0.0395
Citric	0.0870
Succinic	0.00665
Butyric	0.00153
Propionic	0.00137
Acetic	0.00187

3. Monochloroacetic acid is more highly ionized than acetic, and penetrates more easily.

4. With the saturated dibasic acids the order of penetration is: *oxalic* > *malonic* > *succinic*, which parallels exactly that of their ionization; and here again oxalic, the acid of much greater ioniza-

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[12] This would appear to relate the activity of these acids to their lipoid solubility, a view favored by Harvey (1914) and, for other acids, by Vernon (1913), though on insufficient evidence. Their exact relation to the fatty substances of *Chromodoris* cells has not yet been determined.

tion than the other two, is set apart from them by the character of its curve.

5. Hydroxy substitutions, which result in increased ionization, give three clear-cut instances of increased penetrating ability likewise accompanying this change: (a) *lactic* > *propionic*, (b) *tartaric* > *malic* > *succinic*, (c) *salicylic* > *benzoic*.

There consequently seems no escape from the conclusion that ionization is a primary factor in determining cell permeability for acids;[13] if sufficiently great, the degree of dissociation practically controls the situation, and when chemically related acids are considered it fixes their relative penetrating power with precision; if sufficiently low, as in the fatty acid series aside from formic, some factor allied to capillary activity is dominant—though at the same time it is evident that the constitution of the other acids studied is likewise of importance in relation to the make-up of the cell surface and substance. The general result of these experiments is to indicate that while some material other than lipoids, which may be identified as protein,[14] is primarily concerned in permeability (compare Loeb, 1912^b, and Osterhout, 1913, page 141), yet the cell surface must be regarded as complex and totally misrepresented by some current simple theories of permeability, which explain fairly well the conditions of penetration for single classes of substances (compare Loeb, 1912^a, 1914, page 443; Lillie, 1913; Höber, 1914, page 349).

Comparisons with Stimulation.

Series expressing the relative activity of acids as they affect a number of processes might be compared with the data presented in this paper, were it not for the fact that in many cases such a comparison, at least on the quantitative side, would involve the possibility of a serious fallacy. This is particularly true of figures purporting to give the relative toxicity of acids, and the fallacy lies here: the times required for the same concentration of different substances to produce a given effect are commonly assumed to be proportional to the concentrations of these substances which will produce another result within some fixed time; on the contrary, the dilution-*versus*-effect curves for various substances may be of entirely different character (compare Fig. 3), and little correspondence of such acid series could reasonably be expected; moreover, the mechanism of absorption of substances from *very* dilute solutions may be quite different from that at high concentrations (compare, for example, the sense of smell in vertebrates).

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[13] This is also indicated by the results of Dale and Mines (1911) regarding the contraction of muscle in acids.

[14] Shown also by experiments, to be described elsewhere, on the penetration of *Chromodoris* tissues by acid-salt combinations.

(a) I wish particularly to attempt an explanation of sensory stimulation by acids, on the basis of the present data, but a more general case of stimulation may first be considered—that of induced membrane formation in the sea urchin egg (see Loeb, 1913, page 133). The order of acid effectiveness in leading to membrane formation is given, in general, as:

Monobasic fatty acids > dibasic acids > mineral acids,

which is just the reverse of that obtained in considering cell penetration. Within these groups of acids we find stated (Loeb, 1913, page 133) the following approximate order of efficiency (in 0.5 M NaCl solution).

TABLE X.

*Orders of Efficiency in Membrane Production.**

Butyric > *propionic* > *acetic* > formic.
Propionic > lactic.
Benzoic > *butyric.*
Oxalic, tartaric > *citric* > *succinic.*
HNO_3 > $HCl(>H_2SO_4)$.

* The italicized instances agree with the penetration series.

Loeb (1913, page 139) comments upon the apparent disagreement of these facts with the dissociation theory, but points out that it is only the mass of acid which has actually entered the egg that is to be considered, and that the magnitude of this inwardly diffusing mass depends upon the constitution of the acid. But it will be noted that it is only in certain particulars that the membrane and penetration series agree, and that the relative position of the fatty, dibasic, and mineral acids, of formic acid, and of hydroxy substitution in the case of lactic acid, is reversed. Harvey's series for acid penetration, by agreeing fairly well with that obtained for the *Chromodoris* tissue at the same concentration, strongly suggests the validity of my acid series for tissue cells generally, and we must conclude either that the acids leading to membrane formation act merely upon the surface of the egg, which is chemically different from the substance of the interior,[15] or else that the permeability of egg protoplasm for acids is qualitatively different from that of tissue cells. Both conclusions may be correct; but the fact of membrane formation shows that the surface of the mature egg is in its physicochemical make-up different from that of the generality of cells, and the short time in which very dilute solutions of certain acids will bring about membrane formation, as compared with their speed of diffusion through protoplasm, makes it seem certain that the parthenogenetic power of acids involves only a reaction with substances at the surface of the egg, not the egg protoplasm as a whole. This view is not incompatible with Kite's (1913) conclusion that for certain substances the permeability of egg surface and cytoplasm are the same. As far as the evidence for acids is concerned, we must regard the egg surface not as a simple thing, but as quite complex and as containing not only elements which are similar to those of tissue cells,[16] but also others or else the former in different proportions.

(b) Turning now to the matter of sense organ stimulation by acids, it may first be pointed out that the order of permeability to acids for different regions of the body of *Chromodoris* (see Table I)—*viz.*, anterior tentacles, rhinophores > gills > buccal mantle > posterior mantle edge > lateral mantle (under surface) > edge of foot > dorsal body surface—appears to be also the order of the sensitivity of these parts to acids. This statement is made on the basis of work on the behavior of *Chromodoris* by Dr. L. B. Arey,[17] and of independent experiments of my own.

Tests have been made upon marine animals, namely *Holothuria surinamensis*, *Stichopus moebii*, and *Ptychodera* sp., and upon earthworms, which show that, as with human taste, the limiting dilutions beyond which various acids are non-active in stimulation follow this order: fatty acids < dibasic < mineral acids, which agrees in a general way with the penetration series.

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[15] The fact that isolated egg fragments can form membranes is no objection to this interpretation.

[16] Compare the instances in which the penetration series holds (Table X) for parthenogenesis, and the fact that cell-permeability and membrane-formation series are the same for alkalies (Harvey, 1911; Loeb, 1912^c).

[17] I am indebted to Dr. Arey for permission to include certain of his observations in advance of publication.

For more detailed comparison, data obtained from experiments with the worm *Eisenia foetida* may be considered.. These experiments were carried out in the following way:[18]

Each worm (*w*, Fig. 4) was placed upon a low ridge (*r*–*r*) dividing into two compartments (*a*, *b*) a trough constructed of paraffin walls upon a ground glass plate (*p*); compartment *a* contained an acid solution, *b*, water once distilled, to a depth of about 1 cm., each compartment holding 50 cc. of fluid. The anterior end of the worm being immersed in the acid solution, it is stimulated by the acid, and the worm withdraws into the water. The time occupied by this response (from the instant of immersion until that of complete retraction, measured with a stop-watch) is used as an estimate of the amplitude of the reaction due to stimulation by the particular solution,—in other words, as an index of the intensity of stimulation. The figures given are the means of twenty-five tests at each concentration for each acid.

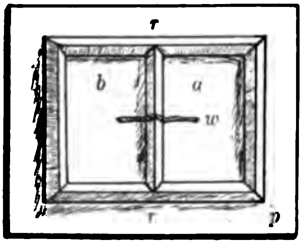

FIG. 4. View of apparatus for stimulation experiments with earthworms.

The time of response so obtained is not strictly a reaction time, as some have termed it;[19] but it is the time required for the performance of a definite act as the result of stimulation,—quite a different thing, and a quantity which we may reasonably hold to be proportional to stimulus intensity.[20] I mean by a stimulus the alteration produced in a receptor,[21] not the concentration (density) of the stimulating agent.

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[18] Details of this method, particularly of the dozen or more recognized sources of error which surround it, and the means taken to overcome and estimate these complicating factors—such as the size of the animals, their previous history, the proportion of their length immersed, etc.—cannot be described here. Posterior stimulation was also studied.

[19] Parker and Metcalf (1906), Hurwitz (1910), Shohl (1914).

In comparing these curves of response (Fig. 5) to several dilutions of a number of acids, the following are the outstanding points and the only ones which will be considered here: (1) Different types of curves are obtained (a) with strong mineral acids,

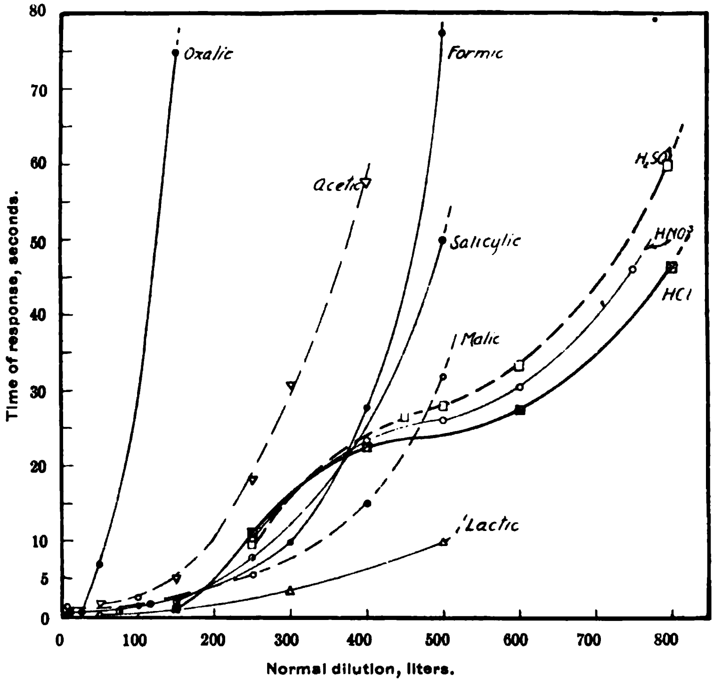

FIG. 5. Time of response to dilutions of nine acids, for the earthworm (see text). No measurable responses were obtained above the dilutions shown.

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[20] Or to the difference between this intensity and a factor expressing the tendency of the worms to progress normally in a forward direction. There are further complexities when the stimulation of the tail end is considered.

[21] In the present case, rather the integral of the partial stimulations experienced by the individual ceptors, according to whatever scheme this integration is accomplished in the nervous system of the animal. Except for the prostomium, there is good morphological ground for the opinion that this scheme may be fairly simple.

and (b) with weak organic acids.[22] (2) The strong acids follow the order of their ionization, but this at such high dilutions that the correspondence probably has little real reference to dissociation. (3) The weak acids fail to stimulate at lower dilutions than do the strong acids. (4) The weak acids are more stimulating in proportion to their ionization than would be predicted if this were the only factor involved.

The points numbered (1), (3), and (4) find their exact counterparts in the penetration series, but the time quantities involved make it obvious that the ceptor surface only is concerned in stimulation. That there is no detailed correspondence in the position of acids in the two series is not surprising, since this worm lives commonly in manure piles, and its chemoreceptors are therefore probably specialized in a peculiar way.

Points (3) and (4) have, of course, long been recognized in taste stimulation (human), and indeed appear universally when chemoreceptors are considered. Various explanations have been suggested to account for these facts, such as the possible greater ionization of the part of the acid at the cell surface, etc.; but it seems to me that explanations of this type cannot hold. The parallelisms between the stimulation series and the penetration series show unmistakably that chemical stimulation involves the solution of the stimulant in the surface of the ceptor cell; and from such solutions or combinations the requisite electrochemical effects can readily be derived (compare Beutner, 1912).

Further evidence in this direction is afforded by the fact that, if the worms are successively stimulated at short intervals (30 seconds), data are obtained such as are shown in Fig. 6. Two aspects of these curves are significant: (1) the temporary lengthening of the time of response after several stimulations,[23] which may be related to the fact that acids tend to produce at first a decrease in permeability (Osterhout, 1914); and (2) the fact that after a certain period the time of response begins to lengthen very rapidly. Now, the speed with which this falling off occurs varies with the different acids, and was found (at 0.02 N to 0.01 N) to follow the order:

Salicylic, oxalic > HCl, HNO_3, H_2SO_4 >
formic > lactic > malic > acetic.

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[22] Oxalic acid was found to be so toxic that its curve cannot be considered with the rest.

[23] Frequently preceded by a heightened irritability.

The agreement of this order with that of cell penetration at these concentrations (Table VII) is striking, and indicates that as the ceptor is entered by acids, its irritability is immediately decreased.[24]

From this point of view it is comprehensible that in secondary sense cells, as of the vertebrate taste bud, we find nerve terminals in connection with the surface of the ceptor, not penetrating its interior.

We must regard this ceptor surface as a complex structure, and thus far the indication is that it contains both fatty substances and proteins.[25]

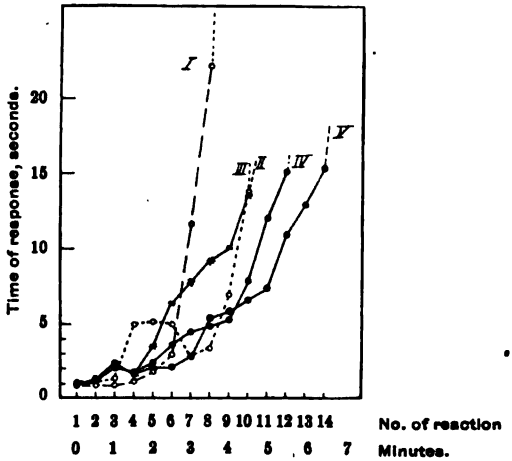

FIG. 6. Effect of successive stimulation on the time of response to 0.133 N salicylic acid; individual worms I to V.

SUMMARY.

1. The method employed for the study of acid penetration depended upon the selection of pieces of tissue from the edge of the mantle of the nudibranch *Chromodoris zebra* Heilprin; these pieces give reasonably uniform and reproducible results when the following conditions are observed: (a) the animals selected are of the same size and of the same kind and degree of coloration; (b) the

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[24] The responses of the earthworm depend upon many scattered chemoreceptors, which are naturally not all influenced at exactly the same time.

[25] Compare Loeb (1912^b), Osterhout (1913), and Loeb and Beutner (1913).

pieces of tissue are taken from corresponding locations, are treated in the same way, and submitted to the action of acids at a constant temperature. This tissue contains an intracellular blue substance, which is turned pink at about $p_H = 5.6$. The time required for this change when immersed in acid solutions is measured under conditions favorable to judgment of the precise moment of color change.

2. The relative penetrating speed of eighteen acids, measured over a range of dilutions, shows that two sets of factors are concerned in penetration. One of these is ionization, the other varies with the constitution of the acid. It is indicated that the cell surface is complex, and contains fatty substances, proteins, and probably other materials.

3. Comparison of these data with observations on artificial parthenogenesis (Loeb) suggests that the stimulus to membrane formation in the sea urchin egg depends upon the union of the acid with the surface of the egg, which is of quite different proportional composition from the cytoplasm of tissue cells.

4. Measurements of the stimulating power of various acids show, in the light of the penetration series, that the surface of chemoreceptors is of complex constitution, probably containing proteins and fatty substances; they show also that the process of stimulation involves the union of the stimulating agent with some constituent of the ceptor surface.

AGAR'S ISLAND, BERMUDA.

The following note was added while this paper was in press. In Harvey's later paper (*Z. physik.-chem. Biol.*, 1914, i, 463–478), which was until a few days ago unknown to me, it is shown that the dilution-*versus*-penetration curves for butyric and hydrochloric acids cut across each other very much as found for strong and weak acids with the tissue of *Chromodoris*. This agreement is rather striking and adds materially to the strength of the interpretation put upon these facts. With the testis epithelium of *Stichopus*, as with the integument of *Chromodoris*, the strong acid penetrates more easily from dilute solutions than does the fatty acid. The same condition appears in Harvey's experiments upon luminous bacteria (*Biol. Bull.*, 1915, xxix, 309). I must therefore disagree with his view that "living cells behave as if they were droplets of oil or fat solvents" (Harvey, 1914, *Z. physik.-chem. Biol.*, i, 476).

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

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59.11.25:49.1

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By Selig Hecht.

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THE WATER CURRENT PRODUCED BY ASCIDIA ATRA LESUEUR[1]

SELIG HECHT

ONE FIGURE

I

1. Ascidia atra is a common tunicate at Bermuda,[2] living attached to rocks, in most cases well under low water. In specimens that were taken to the laboratory and allowed to remain undisturbed until both siphons had opened, a vigorous current could be demonstrated entering the oral, and leaving the atrial siphon. The outgoing current seems always to be stronger than the incoming current, probably because of the somewhat smaller size of the atrial opening, the volume of water passing through the two apertures being, of course, the same (Wallengren, '05, p. 12). This difference in the velocities of the two currents and the angle formed by the axes of the two expanded, diverging siphons, are concerned with keeping the outgoing water from being drawn in again with the incoming current.

I have always found this current to be present until the closing of one or both of the siphons by some stimulus caused it to stop. The oral siphon is the more irritable and is more likely to be closed first, if the stimulus is not very strong. The siphons therefore act like valves, since they control, but in no way help to produce, the current. This is maintained by the cilia which line the lateral margins of the stigmata in the wall of the branchial sac (Orton '13). In Ascidia mentula—a species closely related to A. atra—Herdman ('99, p. 17) has estimated that adults of medium size have 192,000 stigmata. Since there is a row of cilia on each side of these slits, there are in all about 384,000 short rows (ca. 0.2 mm. long) of cilia lashing water through the pharynx of the animal.

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[1] Contributions from the Bermuda Biological Station for Research, No. 42.

[2] My best thanks are due to Dr. E. L. Mark, and to the trustees of the Humboldt Fund, for making it possible for me to stay at the Bermuda Station during the summer of 1915.

Such water currents are not uncommon among marine animals. They have been studied elaborately in Lamellibranchs by Wallengren ('05); and the work of Orton and of Andrews ('93) has shown how similar the mechanism of their production is in Lamellibranchs, Ascidians, and Amphioxus. The details of the maintenance of such currents in the gastrovascular system of Aurelia (Widmark '13) have brought to light the long suspected fact that, unlike Amphioxus, Tunicates, and Sponges (Parker '10 and '14), muscular movements are an important factor in reinforcing the ciliary current produced by medusae.

2. Respiration (cf. however, Dakin, '09, p. 52; Pütter '07, Tab. V) and the gathering of plankton organisms (Orton, p. 20) are the two functions commonly ascribed to the incoming current. To these, Pütter ('07) has proposed the addition of a third. On the basis of metabolism studies, he believes that plankton food is insufficient for the carbon requirement of a variety of marine animals, "and that sea-water constitutes a very dilute nutrient solution, from which they resorb their food entirely or in part" (Pütter, '14, p. 98). To the water current, then, falls the task of bringing this 'nutrient solution' to the place where it may be resorbed.[3]

I mention Pütter's conclusions because he, and others who have criticised him (Lipschütz, Moore et al.) make frequent assumptions as to the volume of the current produced by various animals, having, however, almost no accurate quantitative data on which to base their statements (Pütter '07, p. 293).

The velocity measurements made by Wallengren (p. 23) on the anal current in mussels, and the roughly accurate estimation of the volume of water passing through the Gehäuse of an appendicularian, made by Lohmann ('09, p. 220), show that the quantities are rather large. The only values actually determined are those found by Parker ('14) for a Bermuda sponge. A finger of Spinosella whose volume was ca. 95 cc. discharged 54 cc. per minute through its osculum, or about 78 liters a day.

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[3] An actual case of such a resorption (really a synthesis, too) has just been demonstrated for fats by Churchill ('15) with the fresh water mussel.

II

1. For determining the volume of the current of water generated by Ascidia atra, I adopted a simple indirect method. A glass tube whose bore was a few millimeters less than that of the expanded oral opening, was suddenly introduced into that opening, and the siphon allowed to contract upon it. The tube was then securely tied to the siphon. If an attempt is made to force a tube into a siphon which has begun to close, the mantle tissue is always injured, and the animal never recovers from the injury. The velocity of finely divided carmine particles as they passed through the tube into the animal, was determined at frequent intervals, until a maximum velocity was attained. Ten readings were then made and an average taken. Knowing the diameter of the tube and the velocity of the current, the volume of water passed through the tube may be readily calculated.

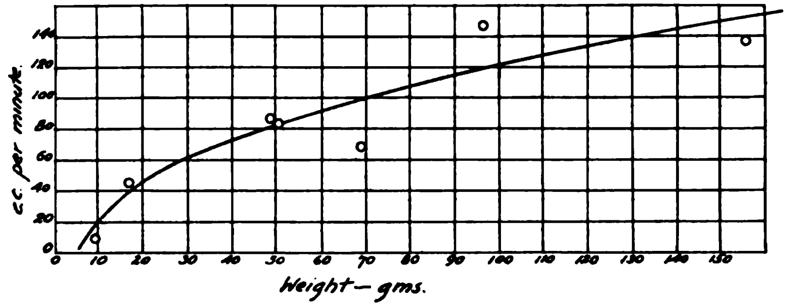

Fig. 1. The volume of sea water passing through different sized specimens of Ascidia atra.

A preliminary determination made on an animal ca. 10 cm. long, gave this volume a value of 94.2 cc. per minute. Seven animals of various sizes were then selected, and the volume of the currents measured. The weights of the individuals and the number of cubic centimeters of sea-water passing through in a minute are given in the accompanying figure.

2. According to these results an adult of medium size (ca. 100 gm.) moves about 173 liters of sea-water in a day. Large as this quantity seems to be, I am convinced that, if anything, it is less than the normal accomplishment. Ascidia atra is not a good aquarium animal, specimens rarely surviving longer than a few days in the laboratory at the summer temperature. The handling and removal from the water consequent on collection and transportation would hardly improve its condition. A case in point is the following experiment.

Experiment 76, July 30

12.30 Fixed the tube into the oral siphon.
Animal kept out of water 3 minutes.
12.45 Average of 10 readings for the time required for carmine particles to travel the length of the glass tube, 3.6 seconds.
1.00 Average of 10 readings, etc., 2.4 seconds.
3.40 Average of 10 readings, etc., 1.1 seconds.
8.25 Average of 10 readings, etc., 0.98 seconds.
9.20 Average of 10 readings, etc., 1.3 seconds.
10.45 Average of 10 readings, etc., 1.4 seconds.

Weight of specimen, 69 grams. Visible length of tube, 5.7 cms. Diameter of tube, 0.50 cms.

It took nearly eight hours for the velocity of the current to reach a maximum, in other words, for the effect of the manipulation at the beginning of the experiment to disappear. I cannot say that recovery was complete, but judging from the appearance and behavior of the animal, I believe that it was nearly so. Recovery therefore is a very slow process. The only experiment which I made with a specimen in its natural environment was unsuccessful, and the termination of my stay at Bermuda prevented its repetition.

3. The above curve shows that the volume of water produced by the current, and hence the total energy of the ciliary mechanism in the branchial sac, is not quite a linear function of the weight of the animal. In general the energy manifested per unit weight varies inversely as the size. That this is true for many invertebrates has been maintained by Vernon ('95) and by Pütter ('07, p. 301) and clearly proved for Octopus vulgaris by Polimanti ('13).

III

Of equal interest with the volumes of water found for the currents in Ascidia, is the pressure at which these currents are maintained. This I determined in the following manner.

A glass tube was tied into the atrial siphon and the animal placed so that the tube was vertical. Enough sea-water was added to cover the animal and the free end of the tube. After a few minutes the oral siphon opened, and a disturbance of the surface of the water near the tube showed the presence of a current through the branchial sac. The level of the water in the jar was then lowered very slowly by means of a siphon, until the height in the tube was about a centimeter below the projecting end. The height of the water in the tube above that of the water in the jar was then measured. The level in the jar was again lowered, and again the height in the tube measured. This was done five times for each animal. Care was taken to see that the oral siphon remained open and submerged during the operation.

After the fifth determination, the animal was removed from its attachment to the tube, the latter retaining its position. The jar was filled with sea-water and similarly five measurements were made of the height of the water in the unattached tube. The difference between the two sets of readings gives the current pressure in millimeters of sea-water.

The pressures thus found are slightly lower than those obtained by Parker for most of the sponges. An average of ten determinations on Ascidia (specimens of ca. 100 gms. each) gives the pressures as 1.7 mm. of sea-water, the range of variation being from 1 mm. to 2.1 mm.

IV

These observations show that the quantity of energy devoted in Ascidia atra toward the maintenance of a sea-water current is distributed, in the light of the functions of such a current, in an efficient manner. A current pressure very much in excess of that necessary to overcome the inertia of the water, would be useless (Meltzer '07). Obviously more is to be gained by utilizing that energy in the moving of large quantities of water through the organism. Such a partition of the energy is actually present.

BIBLIOGRAPHY

Andrews, E. A. 1893 An undescribed acraniate; Asymmetron lucayanum. Studies Biol. Lab. Johns Hopkins Univ., vol. 5, p. 213–247, pl. 13, 14.

Churchill, E. P., Jr. 1915 The absorption of fat by freshwater mussels. Biol. Bull., vol. 29, p. 68–86.

Dakin, W. J. 1909 Pecten. Liverpool Marine Biol. Committee Memoirs XVII. Liverpool, 136 pp., 9 pl.

Herdman, W. A. 1899 Ascidia. Liverpool Marine Biol. Committee Memoirs I. Liverpool, 52 pp., 5 pl.

Lifschütz, A. 1913 Ernährung der Wassertiere durch die gelösten organischen Verbindungen der Gewässer. Ergebnisse der Physiol., Jahrg. 13, p. 1–46.

Lohmann, H. 1909 Die Gehäuse und Gallertblasen der Appendicularien und ihre Bedeutung für die Erforschung des Lebens im Meer. Verhandl. d. Deutsch. Zool. Gesell., Jahrg. 19, p. 200–239.

Meltzer, S. J. 1907 The factors of safety in animal structure and animal economy. Jour. Amer. Med. Assoc., vol. 48, p. 655-664.

Moore, B., Edie, E. S., Whitley, E., and Dakin, W. J. 1912 The nutrition and metabolism of marine animals in relationship to (a) Dissolved organic matter and (b) Particulate organic matter of sea-water. Biochem. Jour., vol. 6, p. 255-296.

Orton, J. H. 1913 The ciliary mechanisms on the gill, and the mode of feeding in Amphioxus, Ascidians, and Solenomya togata. Jour. Marine Biol. Assoc., N.S., vol. 10, p. 19–49.

Parker, G. H. 1910 The reactions of sponges, with a consideration of the origin of the nervous system. Jour. Exp. Zoöl., vol. 8, p. 1–41.

1914 On the strength and volume of the water currents produced by sponges. Jour. Exp. Zoöl., vol. 16, p. 443–446.

Polimanti, O. 1913 Sui rapporti fra peso del corpo e ritmo respiratorio in Octopus vulgaris Lam. Zeit. allg. Physiol., Bd. 15, p. 449–455.

Pütter, A. 1907 Die Ernährung der Wassertiere. Zeit. allg. Physiol., Bd. 7, p. 283-320.

1914 Der Stoffwechsel der Kieselschwämme. Zeit. allg. Physiol., Bd. 16, p. 65-114.

Vernon, H. M. 1895 The respiratory exchange of the lower marine invertebrates. Jour. of Physiol., vol. 19, p. 18–70.

Wallengren, H. 1905 Zur Biologie der Muscheln. I. Die Wasserströmungen. Lunds Universitets Årsskrift, N.F., Afd. 2, Bd. 1, Nr. 2, 64 pp., 3 Taf.

Widmark, E. M. P. 1913 Über die Wasserströmungen in dem Gastrovaskularapparat von Aurelia aurita L. Zeit. allg. Physiol., Bd. 15, p. 33-48.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole**, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. **Gulick**, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. **Mark**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **Barbour**, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. **Linton**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. **Parker**, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts and Sci., **43**(16): 413–455. Apr., 1908.
13. **Cole**, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. **Moeulis**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. **Moegulis**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. **Rand**, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. **Mark**, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting. *Separate:* 6 pp. Nov., 1909.
18. **Smallwood**, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. **Smallwood**, W. M., and **Rogers**, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. **Carpenter**, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. **Esterly**, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. **Cowdry**, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. **Gross**, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. **Smallwood**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23**(4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48**(20) : 737–773, 4 pls. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49**(10) : 569–626, 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16**(3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. Crozier, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. Crozier, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.
41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
42. Hecht, S. — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3) : 429–434. Apr., 1916.

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3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
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9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
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13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
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32. **PARKER**, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. **CROZIER**, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **CROZIER**, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. **CROZIER**, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. **AREY**, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. **CROZIER**, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. **MAVOR**, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. **CROZIER**, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. **CROZIER**, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.
41. **CROZIER**, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
42. **HECHT**, S. — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3) : 429–434. Apr., 1916.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
FOR RESEARCH.

No. 43.

THE RHYTHMIC PULSATION OF THE CLOACA OF HOLOTHURIANS.

By W. J. Crozier.

From The Journal of Experimental Zoölogy, Vol. XX, No. 3.

CAMBRIDGE, MASS., U. S. A.
April, 1916.

Reprinted from The Journal of Experimental Zoölogy, Vol. 20, No. 3,
April, 1916

A LIST OF CORRECTIONS

Crozier, W. J. The rhythmic pulsation of the cloaca of holothurians. Jour. Exp. Zoöl., vol. 20, pp. 297–356, April, 1916.

p. 299, line 18 from top: for "Mr." read "Dr."

p. 305, line 5 from bottom: "oral" should read "anal."

p. 310, line 3 from bottom: for "smoother" read "smooth."

p. 315, the figure on this page lacks *number* and *legend;* the inscription "Duration of pulsation in minutes" is misleading; it should read: "Time subsequent to amputation, minutes."

p. 316, line 2 from bottom: for "table 2" read "table 1."

p. 320, line 2 from bottom: for "convoluta" read "Convoluta."

p. 321, line 8 from bottom: after "intact half" insert "(fig. 20)."

p. 323, line 5 from top: for "(fig. 20)" read "(fig. 21)."

p. 323, line 7 from top: at end of line insert "(fig. 20)."

p. 324, line 8 from top: delete "(fig. 21)."

p. 328, line 2 from bottom: for "variability" read "viability."

p. 332, table 8, line 6: sign "=" should be "±."

p. 333: figure 25 lacks its *number* and *legend.*

p. 335: in legend of figure 27, second line, "5 per cent urea" should read "$\frac{M}{10}$ urea."

p. 348: figure 30 lacks *number.*

p. 352: footnote 20, line 1: for "arrangements" read "arrangement."

p. 306, line 3 from bottom; p. 334, line 3 from top; p. 336, line 3 from bottom; p. 338, line 4 from top; p. 343, line 5 from top; p. 346, line 6 from top; and p. 354, line 10 from bottom: for "1915" read "1915 [?]."

Note—The paper referred to was in type late in 1914; but it is not known when it was issued, if at all.

X. Bibliography 354

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[1] Contributions from the Bermuda Biological Station for Research, No. 43.

I. INTRODUCTION

Holothurians possessing respiratory trees are provided with a cloacal chamber which acts as a pump supplying these organs with sea water. The most obvious manifestation of this activity is the rhythmic opening and closing of the anus. This pulsation has never been studied in detail,[2] though it presents morphological and physiological features of considerable interest.

I have previously (Crozier, '15) brought forward reasons for believing that the cloacal region of a typical aspidochirote holothurian, H. surinamensis, contains within itself the essential mechanism of its pulsation, though there is, superimposed upon this independently pulsating complex, a control of its activity by the animal as a whole. It was found that rhythmic cloacal movements were maintained for about three days in the case of small pieces cut from the aboral end of H. surinamensis, but that in the excised pieces pulsation was continuous and failed to exhibit certain interruptions which are characteristic of these movements in the intact animal. It was noted that Edwards ('09, p. 215) recorded the observation of anal movements in the larvae of H. floridana before the appearance of the respiratory trees. Therefore it seemed that the anal pulsating mechanism might be regarded as constituting another unit in the series of independent effectors, such as pedicellariae and spines, which go to make up the echinoderm neuro-muscular equipment.

The ability of the isolated posterior ends of pedate holothurians to maintain their pulsation for relatively long periods I have further verified by observations upon the following species occurring at Bermuda: Cucumaria punctata Ludw., Holothuria captiva Ludw., H. rathbuni Lamp., and Stichopus moebii Semper.

The problem of the present investigation was to determine the physiological characteristics of cloacal pulsation in holothurians, particularly in the case of the aboral ends removed from the control of the animal as a whole by amputation; and to discover to what extent the laws of rhythmic movement, especially as derived from the study of the medusae, are applicable to these movements in an echinoderm, in which the neuro-muscular conditions are in some respects similar. The account here given is essentially of a preliminary kind. This work has, I believe, resulted in the discovery of a type of material peculiarly favorable to quantitative studies, especially upon the nature of ionic actions.

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[2] The early observations on the pumping activity of the holothurian cloaca are collected by Ludwig ('89–92, p. 387).

The animal principally employed for this study was Stichopus moebii Semper,[3] both because of its availability in unlimited quantity, and since its large size greatly facilitated operative experiments. Unless indication is made to the contrary, it is to be understood that in the subsequent pages this species is always referred to.

To Prof. E. L. Mark I am again indebted for the privileges of the Bermuda Biological Station and the abundant fauna to which it gives access. The work was carried out during the summer of 1914. I wish to record, in addition, my obligation to Mr. L. B. Arey for much incidental assistance. Prof. G. H. Parker has read this manuscript, and has materially aided me both by inspiration and by advice, for which my best thanks are due.

II. PULSATION IN THE INTACT ANIMAL

a) *Morphology of the pulsating parts.* The parts concerned in pulsation will first be described, and, following this, their normal operations, as a basis upon which to interpret the experiments.

The general relations of the contractile parts in the aboral end of Stichopus will be evident from figures 1 and 2. As is necessary in any pulsating system which does not include an elastic supporting structure against which the muscles can work, there are essentially two sets of opposed muscles: 1) the radiating muscles, producing cloacal expansion, and 2) the circular

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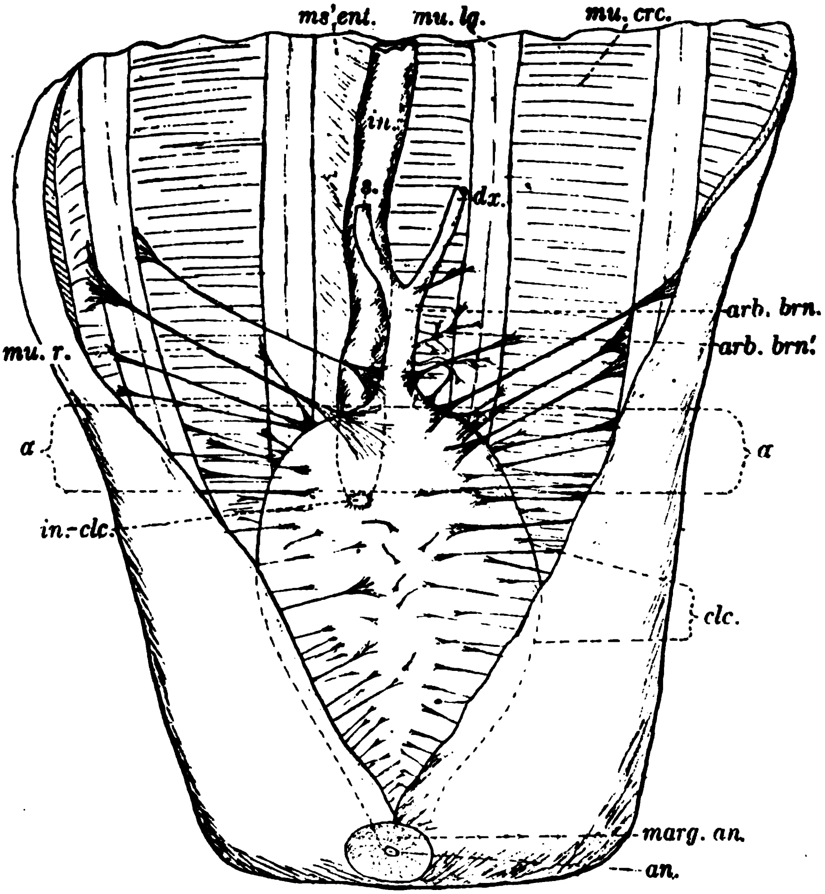
[3] I agree with Clark ('01) that the black, spotted, and uniform reddish-brown forms of this Stichopus are specifically identical. They are physiologically indistinguishable, except in the matter of pigment formation, and in this all intergrades were found among the approximately 2000 individuals examined.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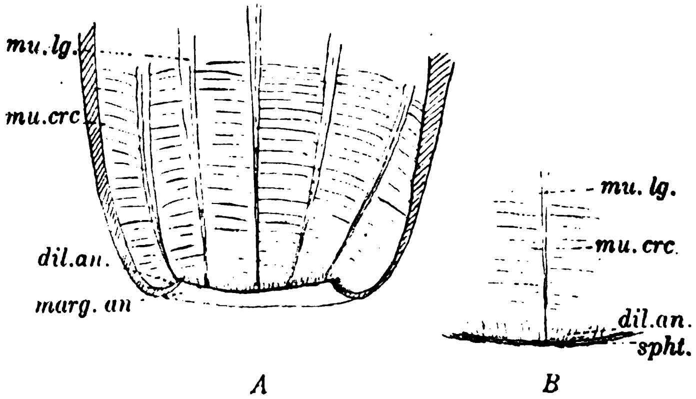
mu. lg.
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dil. an.
spht.
A
B

2

muscles of the cloaca, sphincter, and body wall, producing contration of the cloacal region.[4]

The conditions are, however, much more complex than this simple statement indicates, and it is necessary to consider the distribution of the muscles more in detail. The third limb of the intestine opens into the cloacal chamber (*in.-clc.*, fig. 1) on its ventral side. This opening is guarded by a sphincter, which is kept closed except during defecation. At its anterior end the cloaca gives rise to the stalk of the respiratory trees, also guarded

Fig. 1 The posterior end of Stichopus moebii opened along the mid-dorsal inter-radius; the body wall is somewhat distorted, so that only four of the five longitudinal muscles are seen. From a living preparation (× 2½).

an., anus.
arb.brn., trunk of respiratory tree.
arb.brn'., small derivatives of the respiratory trees, originating rather far down the stalk. When full of water these small branches pass backward into the space about the posterior end of the cloaca.
arb.brn.dx., right respiratory tree stem.
arb.brn.s., left respiratory tree stem.
clc., outline of cloaca.
in., intestine.
in.-clc., union of intestine with the cloaca.
marg.an., anal brim.
ms.'ent., mesentery.
mu.crc., circular muscles of the body wall.
mu.lg., longitudinal muscles of the body wall.
mu.r., radiating muscles of the cloaca. Some of them are shown cut. Only a few of the many strands present are drawn. Some of the strands are of connective tissue, and are not contractile.
α——α, level of amputation in experiments with the isolated cloaca, showing the range of this level.

Fig. 2 *A*. The body wall of Stichopus moebii divided along the dorsal interradius, and the cloaca removed (× ⅝). *B*. A portion of the anal brim flattened out (× 1⅔).

dil.an., dilators of the anus.
marg.an., anal brim.
mu.crc., circular muscles.
mu.lg., longitudinal muscles.
spht., circular fibers of the sphincter.

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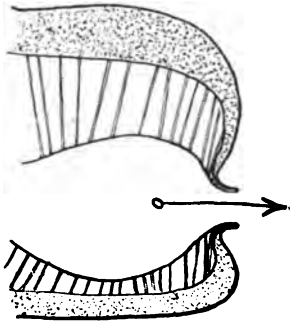
[4] Jordan ('13, '14) has dealt with the contractile properties of the so called 'connective tissue' of the skin of holothurians, and states his belief that this tissue by its degree of slow tonic contraction acts in conjunction with the locomotor muscles. The pulsation movements which we are considering are, as compared with the progression movements, very rapidly executed, so that (aside from their rôle in maintaining a tense support for the radiating muscles) these contractile elements of the integument are not discussed in detail. The relation of salts to their degree of tonus will be studied in a subsequent paper.

by a sphincter (Bordas, '99). Posteriorly the cloaca and body wall are continuous in the region of the anal sphincter. The longitudinal muscles of the body wall are reduced to very fine threads just before they reach the anal sphincter, but there are, in addition to the circular fibers of the sphincter, certain contractile elements set perpendicularly to them. This last mentioned set of muscles assists in the opening of the sphincter; its members are homologous to the radiating muscles of the cloaca.

Concerning the innervation of these structures it is difficult to get precise information, since the nervous elements are refractory to ordinary neurological technique. I was unable to secure methylene-blue preparations in the case of Stichopus. The cloaca is referred to (e.g., Haanen, '14, p. 223) as of ectodermal origin, in agreement with which is the fact that the inner cloacal surface shows, though to a lesser degree, the pigmentation of the outside of the animal. It is, therefore, possible that the cloaca (including the radiating muscles [?] and sphincter) is innervated from the radial nerve strands (Ludwig, '89–92, p. 70), in common with the body-wall muscles. The gut of holothurians is supplied with special nerve strands from the nerve ring, and the cloaca may receive a nervous supply from this source. The holothurians have no aboral nerve ring.

With reference to the finer histological condition of the nervous apparatus, Bethe ('03, p. 22) remarks that "über die histologischen Verhältnisse des Nervensystems ist bei den Echinodermen so gut wie Nichts bekannt," but it seems certain that a subepithelial plexus is present in the form of a true nerve-net (Jordan, '14, p. 380). This would appear to be the condition in many autonomous organs of invertebrates, and especially in structures which exhibit rhythmic contraction (Alexandrowicz, '13).

b) The sequence of movements. Peristaltic movements appear in the intestine, respiratory trees (as noted by Henri, '03), cloaca, anal sphincter and body wall of Stichopus. All but the first of these organs are concerned with the transport of water. In ordinary inspiration the movements appeared in the following order: 1) the anal sphincter opened, 2) a wave of opening moved anteriorly along the cloaca, 3) the stalk of the respiratory trees opened when the wave had run to this level, with the result that the whole cloaca was now wide open; 4) the anus then closed, and 5) the body in the region of the cloaca, which had previously increased in diameter, now began to contract in such a way as to indicate that a wave of constriction was passing forward on the cloaca. This does not mean that the anal sphincter is the seat of origin of the pulsation wave, but is merely a description of the pulsation as seen from the outside of the animal. When the anus was closed, and especially immediately after its sphincter had contracted and the constriction of the cloaca and body wall were increasing the pressure on the fluid within the cloacal chamber, the edge of the sphincter appeared to be acting after the manner of heart-valves, as indicated in figure 3. This was verified by the inspection, from the cut end, of amputated cloacae. Yet, when the cycle of movements began again with the opening



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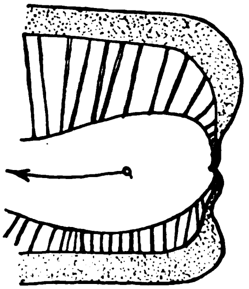

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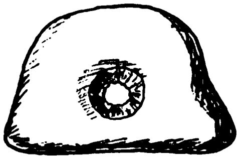

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Fig. 3 Diagramatic longitudinal section through the cloacal end, showing the relation of the sphincter during the pumping of water into the respiratory trees. Arrow indicates the course of the water.

Fig. 4 Showing the form of the anal brim during spouting. The arrow gives the direction of the current.

Fig. 5 View of the posterior end of Stichopus ($\times \frac{5}{12}$). The action of the anal dilators is shown by the irregular form of the edge of the brim.

of the sphincter, its edge was pulled back sharply, a process which was also evident at the termination of spouting (fig. 4) and subsequently; this is the function of the minute muscles above described, which are placed on the coelomic face of the anal brim perpendicularly to the circular constrictors of the sphincter. The opening of the sphincter therefore involves not only the relaxation of the circular muscles but also the contraction of a set of antagonistic dilators, whereas in closing the action of these sets of muscles is reversed. During the opening of the anus the edge of the sphincter showed very clearly the action of the dilator muscle strands (fig. 5). The closure of the brim was usually not quick enough to catch all the water within the cloaca at the end of an expansion period, because the general contraction movement slightly preceded the constriction of the sphincter, so that some of the contained water escaped before the brim was completely closed. The time relations of the phases of a single pumping movement are indicated by the measurements summarized in table 1.

TABLE 1

Time relations of the phases of anal pulsation, in seconds

PHASE	LENGTH OF STICHOPUS	
	20 cms.	30 cms.
(1) Sphincter open	2.5–3.5	3.2–4.0
(2) Sphincter closed	2.4–2.8	2.6–2.9
(3) Expiration	9–10	13–15

In all the measurements of pulsation rate given in this paper, a complete 'pulsation' begins with the opening of the anal sphincter and ends with the start of the next succeeding opening movement. In practice this interval could be timed with extreme accuracy, since, immediately the anal sphincter began to open, the whitish interior of the cloaca became visible in the center of the dark brown or black field presented by the general surface of the animal. The slowness of the movements also greatly aided precision in their measurement. Five to ten pulsations were timed with a stop-watch, and the result reduced to "time for ten pulsations;" where less than 10 successive pulsations could be timed, the observation was repeated two or three times; closely agreeing times were always obtained in successive determinations.

When the cycle of movements comprising a single pumping operation had been carried out some 6 to 10 times in succession, an interruption occurred, in the form of a wide opening of the anus persisting for some seconds (table 2), during which the water previously pumped in was forced out of the respiratory trees. The ratio of the number of inspirations to an expiration varied directly with the size of the animal.[5] This expiration, or 'spouting,' was accompanied by retraction of the tentacles, constriction of the buccal sphincter, and a general body contraction. The interesting point in this connection, however, is that when the central edge of the anal sphincter was watched closely it was seen that the sphincter continued to pulsate at a normal rate, though with greatly reduced amplitude, in spite of the fact that it was being held wide open by the body-wall muscles and the strongly contracted radiating strands. The first inspiration succeeding a spouting act was usually of greater vigor than the following ones.

TABLE 2

Stichopus moebii; occurrence of spouting in relation to the number of cloacal pulsations between each expulsion of water; / indicates an expulsion of water

LENGTH	TIME FOR TEN PULSATIONS	NUMBER OF PULSATIONS BETWEEN SPOUTING	AVERAGE NO.
cms.	*seconds*		
19	54	5/6/6/6/7/5/8/5	6.0
23	66	6/7/6/8/6/10/6	7.0
25	60	9/8/10/9/7/8/10	8.5
30	75	9/10/10/11/8/7/9/10	9.2

The pumping movements also ceased, the oral sphincter contracting firmly, when the posterior end of the animal was stimulated tactually, especially when the papillae were touched. Stimuli of various kinds applied to the animal's surface resulted in reactions which included the temporary constriction of the anal sphincter.[6] Closure of the anus was also a preliminary operation when Stichopus was beginning to engage in locomotion.

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[5] A similar observation was made by Hérouard ('89, p. 689).

The control of anal rhythm by the animal as a whole includes, then, (1) the cessation of pulsation by causing the anus to constrict (either in response to stimuli, or in locomotion), and (2) the great restriction of the amplitude of pulsation (in 'spouting') due to the contraction of the radiating muscles and the muscles of the body. Of these modes of control only one, contraction in response to stimulation from the outside, persists in the isolated cloaca.

As regards the function of cloacal pumping in holothurians, it is clear from the work of Bordas ('99) and Winterstein ('09) that the water drawn in and out of the respiratory trees serves to supply the coelomic fluid with oxygen, to remove excretory products, and at the same time to control the amount of fluid contained within the animal, upon which its locomotor movements depend.[7] In connection with the respiratory function of pulsation it is of interest to note that if by repeated mechanical stimulation, pulsation is prevented from occurring for some minutes, the movements which occur when pulsation is resumed are not increased in rapidity, but are of greater amplitude than those normally seen in undisturbed specimens. This was also noted by Pearse ('08, p. 272) in the case of Thyone, and he drew the conclusion that the animal was in this way making up for its preceding oxygen deficiency. This deduction by no means follows, however, because when repeatedly stimulated holothurians decrease greatly in volume (cf. Crozier, '15) by expelling water from the anus, and when left undisturbed after such stimulation they tend to return to their original size. In doing this the first pulsations of the new series are more vigorous than usual, as is also found after each normal water expiration. The increase in amplitude observed after forced cessation of pulsation is therefore only remotely connected with respiration; this view is supported by the low oxygen requirements of echinoderms, to be considered subsequently, and the known presence of other modes of respiration than that associated with the respiratory trees. The phenomenon of increased amplitude of pulsation subsequent to forced inactivity in a pulsating structure is also well known in the vertebrate heart, and may be seen, for example, in such curves as those plotted by Vernon ('10) to show the recovery of the heart-beat after perfusion with protoplasmic poisons.

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[6] These reactions were similar in every respect to those of the previously described H. surinamensis (Crozier, '15 [?]).

[7] The movements of holothurians have recently been analyzed by Jordan ('14) from the standpoint of his conception of a 'Hohlorgänartig' animal.

The occurrence of anal rhythmic pulsations in holothurians is not an isolated phenomenon, for such movements (with a suggested similar function) occur among Enteropneusta (Willey, '99, p. 244), both in the adult[8] and in Tornaria (Willey, '99, p. 306), and in decapod crustacea (Miller, '10), etc. In the lobster and crayfish the muscular arrangements for producing anal rhythm are in a general way similar to those in holothurians, since in the former case circular muscles and radiating muscles running to the body wall also occur; furthermore, the coördinating mechanism is a local one (Miller, '10, '12).

c) *Correlation with size.* It has long been a matter of general knowledge that the activity of animals varies with their size, smaller animals being more active than larger ones. This rule holds conspicuously for the execution of rhythmic movements by animals of the same species. The rapidity of breathing movements, etc., are not, however, simply proportional to the reciprocal of length or any other body measurement, but the empirical curve expressing the relation between size, or weight, and activity is almost invariably of a rather complex hyperbolic type. Polimanti ('13) has recently reviewed the literature of this subject, and has supplied an additional example in the respiratory movements of Octopus. The equation derived from his data is of the form:

$$y = a + bx + cx^2 + \quad . \quad . \quad . \quad .$$

where y = weight, and x = respiration rate.

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[8] I have observed that the amputated posterior end (4–5 mm. long) of the Bermudan Ptychodera sp. will pulsate in sea water. A sphincter ani is present.

The rate of rhythmic pulsation of the anal sphincter for animals of different sizes was observed in Stichopus, Holothuria surinamensis, H. captiva, and Cucumaria punctata. The results are given in figures 6 to 9. The data in the case of Stichopus (fig. 9) are more irregularly distributed than with the other

Relation between size and pulsation-rate

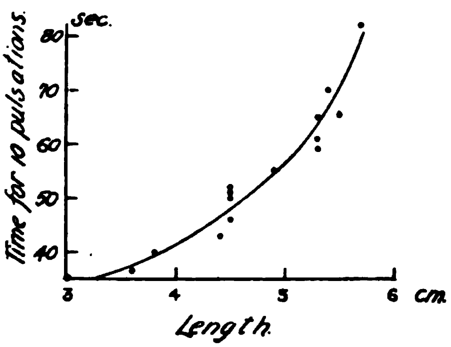

Fig. 6 Cucumaria punctata. Temp. = 24.5°

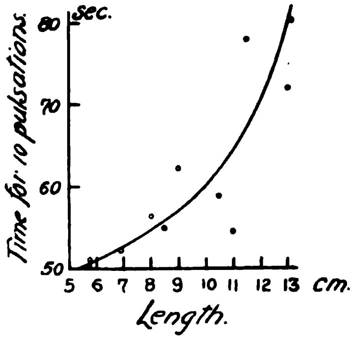

Fig. 7 Holothuria surinamensis. Temp. 25.0°

forms. This is due to the fact that in the instances measured the individuals of the other species were of more homogeneous history, in that their small size made it possible to keep numbers of them in a single aquarium, so that their pulsation rates were all measured at one time. Because of their bulk the specimens of Stichopus had to be studied separately, often at intervals of some days, so that various factors, including temperature differences, probably entered, tending to lack of uniformity in the results.

Relation between size and pulsation-rate

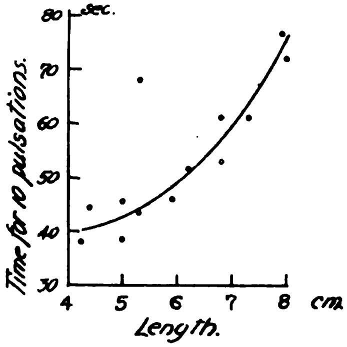

Fig. 8 Holothuria captiva. Temp. = 26.0°

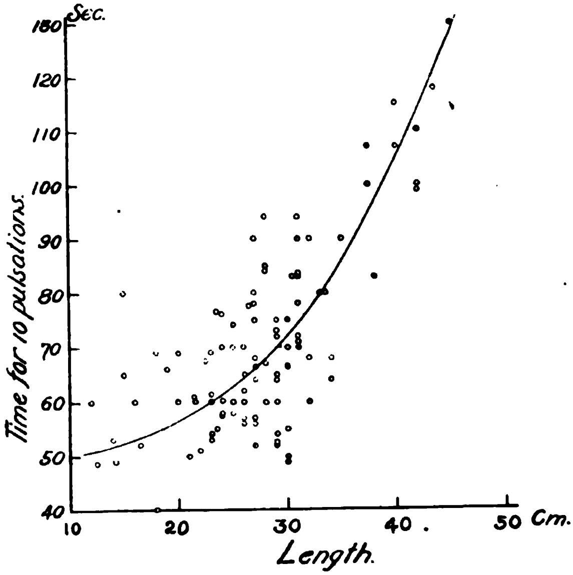

Fig. 9 Stichopus mobii. Temp. = 24°-26°

The smooth curves in figures 6 to 9 are in a general way similar to that of Polimanti ('13) for the rate of respiratory movements in Octopus. The weight of Stichopus is a simple function of length, such that

$$\text{Weight in grams} = (0.034 \pm) \times (\text{length in cms.})^3$$

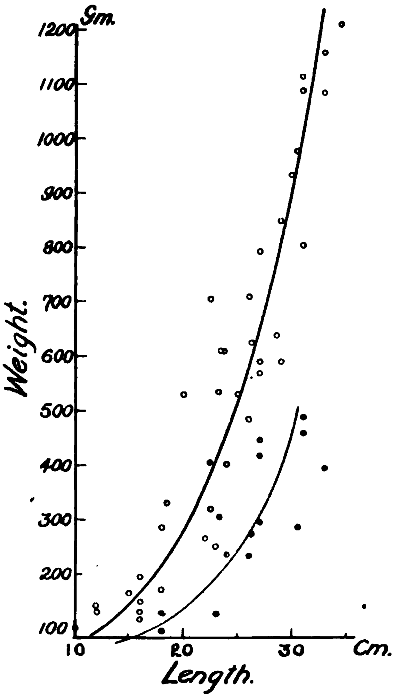

Fig. 10 Relation between weight and length in Stichopus: —○—○—, weight including that of the body fluids; —●——●—, weight of the integument alone.

(fig. 10); this type of relation between weight and length is also true for the other species. If pulsation-rate were plotted against weight the resemblance to Polimanti's curve would be increased. As shown in figure 10, the weight of the integument alone is too variable to give smoother results; the variation is produced mainly by the fact that the skin seems to absorb water to a varying extent, depending upon its rigidity. The curves relating size to pulsation-frequency are of the same type as that found by plotting Mayer's ('06, p. 8) data on diameter vs. pulsation-rate in Cassiopea.

In attempting to account for the relation of pulsation-rate to size, it is possible to adopt the view that the rapidity with which an animal executes a given act is a measure of the amount of energy available for the performance of that type of work, and that when operating under similar conditions the relative energy content of different individuals in the same species may in this way be compared. According to this interpretation, larger animals must contain less motor energy, proportionately, than do smaller ones of the same kind. The observations of Tashiro and Adams ('14), that the cardiac ganglia of large Limuli have a lower output of CO_2 per gram of nerve-substance than do the (smaller) corresponding ganglia of smaller Limuli, and those of Child ('13, p. 140), who found by the KCN method that young (small) individuals of Planaria had a higher rate of metabolism than larger ones, lend support to this general idea.

III. COÖRDINATION OF THE PULSATING COMPLEX

The pulsation of the cloaca exhibits a high degree of coördination in the action of a number of individual effectors. The questions arise, From what center, if any, does the stimulus to pulsation proceed, and by what means are the various muscles brought to act in appropriate sequence?

The following observations and experiments bear upon the answers to these questions. The points to be considered are (1) the cycle of pumping movements in the cloaca, and (2) the cessation of these movements, with the exception of those of the anal sphincter, during the expulsion of an expiratory stream. It may be mentioned here that during defecation the sphincter movements are not interrupted, and as a result the faecal masses present beaded constrictions at regular intervals along their length, the constrictions (fig. 11) being formed by the pressure of the sphincter as it attempts to close. This applies to Stichopus and Holothuria; but in Cucumaria there are no definite castings, because the ejected material is discharged in fragments during the course of an expiratory act.

a) *Effects of autoevisceration.* When Stichopus undergoes autoevisceration the cloaca ruptures in the region of the termination of the intestine, and the gut is passed out, pulling with it more or less of the respiratory trees; the whole then being autotomised, the animal which remains consists of the dermo-muscular tube and the cloaca. Such an animal continues to exhibit cloacal pulsation, the water, however, being now pumped into the body-cavity directly. The rate of pulsation in eviscerated animals was normal, up to at least twelve hours after autotomy, and the characteristic interruption of the inspiratory movements after every sixth to tenth one, by the expulsion of water, continued as

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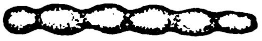

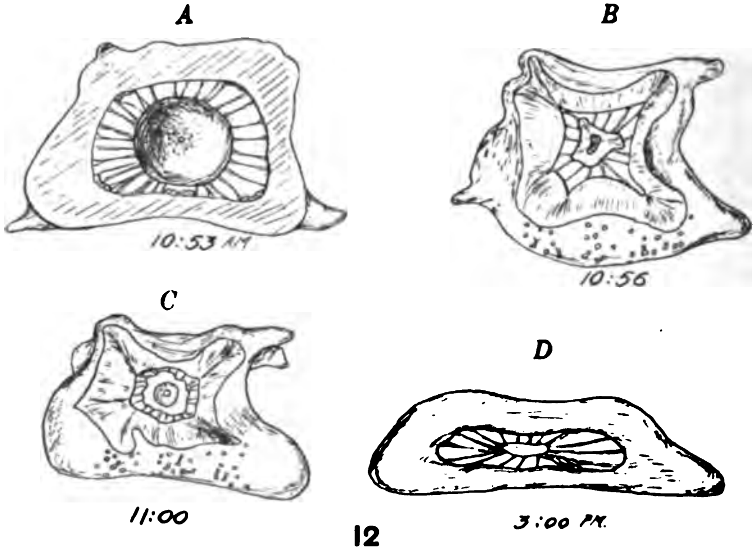

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Fig. 11 A casting of Stichopus ($\times \frac{5}{12}$), showing the constrictions, somewhat exaggerated, due to the continuance of anal rhythm during defecation.

Fig. 12 Stages in wound closure, drawn from one preparation, at the periods indicated.

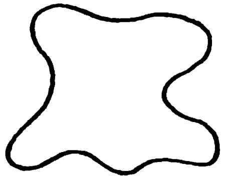
before evisceration. The general tonal depression of the animal involved some decrease in pulsation amplitude.

The stimulus to spouting, then, does not necessarily originate from a state of tension in the full respiratory trees.

b) *Effects of amputation.* The cloacal end of Stichopus when amputated at the level α——α (fig. 1) contracted firmly. But if it was then put into sea water, or into one of a variety of salt solutions subsequently to be described, it very shortly opened up and continued to pulsate rhythmically, though with a gradually decreasing frequency and amplitude, for as much as thirty hours. The duration of pulsation depended upon the size of the excised piece, the volume and composition of the surrounding solution, and the existing temperature.

About one minute after excision the cut edges of the body wall began to bend inward (fig. 12 *B*). The cut edge of the cloaca itself was flared outward, while its lumen was closed by the contraction of a powerful circular muscle about midway between respiratory trees and anus. The radiating muscle-strands near the plane of the cut were relaxed, but they contracted when pinched. The flared anterior end of the cloaca was then pulled toward the anus, while the cut edge of the body wall was bending inward. These processes, tending to close the remnant of the coelom, had a highly protective appearance. Some three to four hours after the inbending of the edge of the cut, the body wall in this region became flabby and relaxed, and a progressive degeneration, which involved swelling and mucoid disintegration, began at the cut edge. After about five to six hours the interradii became sunken inward, so that a cross section of the pulsating piece had the appearance shown in figure 13; in addition, the previously inturned edges of the body wall were now relaxed. At this time, that is after about six hours' isolation in sea water, the excised cloacae looked like the one sketched in figure 14.

The history of individual preparations, made in the way described in the preceding paragraph, was followed under various conditions until they ceased to live. I shall refer here merely to the performance and fate of cloacal pieces contained in one liter of sea water. The time required for the execution of 10 rhythmic



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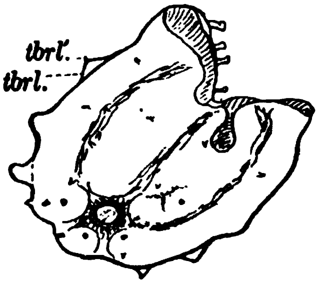

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Fig. 13 Cross-section of a cloacal piece, showing the sunken condition of the inter-radii.

Fig. 14 Sketch of a pulsating piece, from above (× $\frac{5}{12}$).

tbrl., one of the characteristic wart-like tubercles.

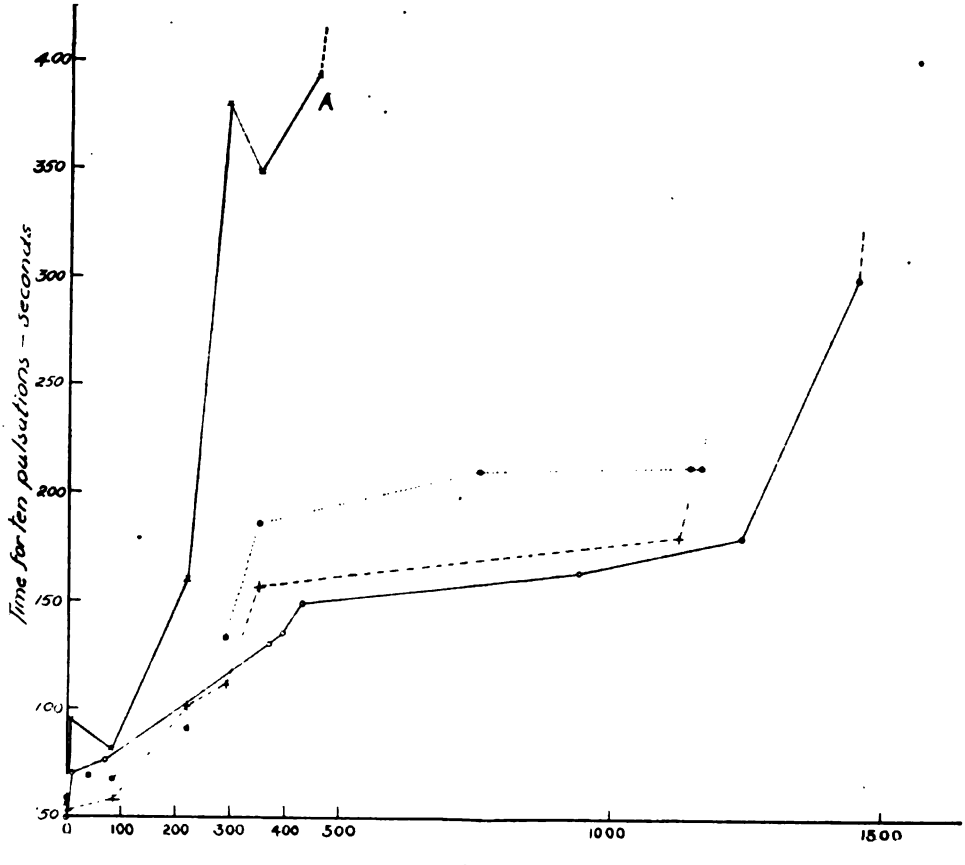
tbrl.', the delicate tactile papillae which they bear (not noted by previous writers).

movements of the anal sphincter was measured at intervals of time subsequent to the isolation of the pieces. Exhaustion curves obtained in this way are illustrated by figure 15. A typical example is given in detail:

Experiment 33.3. June 21. *Stichopus moebii;* 24 cms. long; normal pulsation, 58 seconds for 10 movements. Temperature varied between 26° and 24°C.

MINUTES ELAPSED	SECONDS REQUIRED FOR TEN MOVEMENTS	NOTES
0		Cloacal end amputated; put in 1000 cc. sea water
2		Sphincter opening; beginning to pulsate
5		Pulsation becoming more regular
40	81.0	Amplitude fuller
85	68.5	Normal
220	91.5	
290	133.0	
350	176.0	Closure no longer complete
755	210.0	
1145	212.5	
1165	212.0	Barely pulsating. Soon stopped

There were in general two modes of exhaustion; one, a rapid type (marked 'A' in fig. 15), the other showing a sort of 'fatigue level.' The first type of exhaustion was exhibited by pieces of shorter length than that of the other 'normal' pieces, i.e., cloacae cut off below the level α——α (fig. 1). It is possibly of significance that the earlier part of these exhaustion curves bears a superficial similarity to those of autocatalytic reactions, which might be taken to indicate that there is here an autocatalyst of exhaustion, represented by fatigue products. The sharp upward bend of the curves near the time of cessation of pulsation indicates that pulsation continues until some critical condition is arrived at within the pulsating mechanism, beyond which spontaneous rhythm is not possible. The exhausted condition was



not reversible experimentally, though sphincters which had ceased to beat rhythmically in sea water would pulsate once in response to a touch or to the local application from a pipette of a small volume of a stimulating solution, and they could momentarily be revived by immersion in sea water free of Ca; possibly the mechanical stimulation involved in the last experiment was responsible for part of the pulsation.

A very significant fact about the pulsation exhibited by the isolated cloacal ends, is that the rhythm was not interrupted at intervals corresponding to the expulsion of the cloacal stream in the intact Stichopus, but was indeed perfectly continuous, unless complete contraction was induced by some especially applied stimulus. This condition is intelligible in view of the fact above noted that in the normal animal the edge of the sphincter was observed to pulsate with faint amplitude during spouting. It follows that the stimulus to spouting has its origin outside the cloaca.

The early parts of the exhaustion curves show a peculiarity which must be noted. Immediately after amputation and immersion in sea water, the contracted musculature of the cloaca began to relax, and by two minutes, at most, had begun to open. Very commonly it then remained wide open for some seconds, and when pulsation was resumed the rate of sphincter movement was very slow and closure incomplete. The rate of movement was soon improved, however, coincident with the institution of contractions and relaxations of the cloaca, which were of maximal amplitude. It would seem that the coördination of the members of the pulsating complex is disturbed by cutting this complex away from the rest of the animal, and that some little time must elapse before harmonious interaction can again be established. The full amplitude of the pulsation was usually revived very suddenly.

Rhythmic pulsation in the isolated posterior ends was similar in all essential respects to that in the intact animal. The time relations of the several phases of movement are given in table 3, which may be compared with table 2, showing the normal condition in the intact Stichopus. In the isolated pieces it could be observed that the cycle of movements constituting a complete pulsation began at the cut end of the cloaca.

TABLE 3

Time relations, in seconds, of the phases of pulsation for the isolated cloaca in sea water

EXAMPLE	1	2
Open phase	5.6–7.3	7.0–8.0
Closed phase	3.0–5.7	5.2–5.6

The direction of the current of water produced by the isolated cloaca was studied with the aid of carmine suspended in sea water, and also by small 'flags' of mucous or bits of thread attached to the inner edge of the sphincter. The current was directed anteriorly, as in the intact animal. The maximum fluid pressure developed in the amputated pieces was ascertained by inserting a glass tube of appropriate size into the anus, which then contracted tightly about the tube. The pressures were never more than 1 cm. of sea water. In the intact animal the cloacal pressure is much greater, since the body muscles then play a greater part in the pumping.

The pulsating sphincter exhibited a refractory period, such that if the sphincter were touched at the very beginning of an opening movement, it continued to open to its normal extent; but if touched at the edge when more than one-half open, the brim closed down promptly, though some local retraction was evident at the point stimulated (fig. 16).

It was pointed out in a previous section that the rate of cloacal pulsation was correlated with the size of the holothurian. It was important to learn if this correlation persisted in the excised posterior ends. The rate of pulsation in cloacal pieces of equivalent size (i.e., amputated at the level α——α in fig. 1) derived from 10 Stichopi of increasing lengths is tabulated in table 4, from which it will be seen that, though some trace of this general effect may be maintained, it is by no means clear cut; the rate of pulsation in the isolated posterior ends was very rapidly reduced to an approximately uniform level soon after excision.

TABLE 4

Relation of pulsation rate in the excised cloacae to the size of the animals from which they were obtained. Temperature = 26.0°

NO.	LENGTH	TIME FOR TEN PULSATIONS	
		Before amputation	After amputation
	cms.	*seconds*	*seconds*
1	23.5	55.3	88.6
2	24.0	58.0	70.1
3	26.0	64.3	88.0
4	27.0	80.0	90.2
5	28.0	84.1	100.2
6	28.3	85.0	89.5
7	29.0	65.4	85.0
8	30.0	70.0	84.3
9	31.0	78.5	86.4
10	31.0	83.0	80.8

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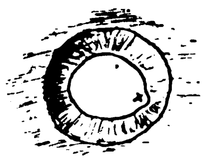

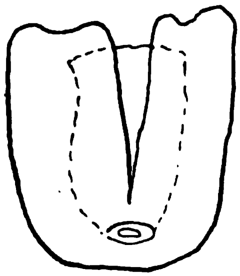

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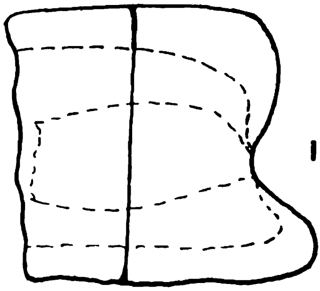

18

Fig. 16 Reaction of the opening sphincter to tactile stimulation at the point x (× 1$\frac{2}{3}$).

Fig. 17 For explanation, see text.

Fig. 18 For explanation, see text.

The rate of rhythmic movement is, however, very much conditioned by the length of the cloaca included in the cut off piece. In table 5 is given a summary of the history of three posterior ends of different lengths cut from animals of the same size, which illustrates this point.

TABLE 5

Dependence of pulsation rate and duration upon the length of the excised piece. A = *complete cloaca;* B = *about three-fourths of cloaca;* C = *one-half of cloaca; each from a Stichopus 26 cm. long*

TIME ELAPSED SINCE AMPUTATION	TIME FOR TEN PULSATIONS		
	A	B	C
minutes	*seconds*	*seconds*	*seconds*
10	71	93	115
70	81	90	123[1]
250	99	125	∞

[1] Irregular movements.

This suggests that the stimulus to pulsation originates in the anterior part of the wall of the cloaca with its associating radiating muscles, in the sense that the structure which of itself pulsates in sea water faster than any other part of the cloaca is located there, and that its activity carries with it that of the other contractile parts. A somewhat parallel case is found in the vertebrate intestine, where the transmission of pulsation depends upon the myenteric plexus (Cannon and Burket, '13); Alvarez ('14) found that the rate of pulsation of strips from the small intestine varied inversely with the distance from the pylorus. The localization of the origin of pulsation in the cloaca of Stichopus will be further considered later in this paper.

Implied in the above description is the view that the fastest beating member of a pulsating complex determines the rate of movement of the whole. This idea was put forward by Loeb ('00 a, p. 29) in his explanation of the reversal of pulsation in the tunicate heart; the neatest demonstration of the operation of this principle is probably found in an experiment of Mayer ('11, p. 7), who grafted together a large and a small Cassiopea and found that the faster beating medusa determined the pulsation rate of the whole mass.

The possibility presents itself that the stimulus to pulsation normally arises outside the cloacal region, and that the cut-off pieces continue to pulsate from a sort of 'habit' or 'organic memory.' It, indeed, is possible to impress rhythms upon holothurian structures, as I have discovered in certain experiments upon the physiology of the 'shading reflex.' Shadows were cast upon the anterior end of a Holothuria surinamensis at 0.25 minute intervals, to which the animal reacted (cf. Crozier, '14, '15) by more or less complete retraction of the tentacles and contraction of the buccal sphincter; after the 115th successive stimulation and reaction, the rhythmic shading was discontinued, but the animal *continued to retract the tentacles, etc., at very nearly 0.25 minute intervals for the next succeeding 3 minutes* (i.e., 11 times), after which the 'reactions' became of irregular occurrence. This observation was repeatedly confirmed.[9] But the fact that the rhythm of the isolated anal parts could be caused to stop, by appropriate sensory stimulation, and then to resume again in perfectly reversible fashion, argues against this interpretation, as does indeed the whole behavior of the pieces in different salt solutions.

c) *Mutilation experiments.* Stichopus having the oral end, including the nerve ring, amputated, continued to exhibit rhythmic anal movements like those of the whole animal. As long as the new anterior end remained closed by the close approximation of the inturned edges of the cut, so that some internal fluid pressure could be produced, the typical spouting movements also occurred. Animals with the anterior end excised, however, rapidly lost tone and, in the case of Stichopus, died within a day or so. With Holothuria surinamensis, as appears also to be true of Thyone (Scott, '14, p. 289), anal rhythm becomes slow and weak soon after the amputation of the anterior end. This is associated with a general loss of tone and a totally quiescent condition, which is only removed upon the regeneration of a new anterior end (Crozier '15 [?]). The stimulus to 'spouting' is therefore probably derived from a condition of general body tension, resulting from the pumping of water into the interior of the body when the muscular integument presents a volume of definite size. The stimulus is not, necessarily at least, conditioned by a state of tension in the respiratory trees alone, since the eviscerated animals behaved in this respect as did the complete ones.

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[9] Quantitative investigation of this phenomenon is contemplated, and should provide important data relative to the physico-chemical nature of 'protoplasmic memory.' Somewhat comparable rhythms of short period, impressed by experiment, are not unknown among plants. It is especially to be noted that the condition here described in Holothuria is one of 'positive memory,' in contrast to the 'negative memory' [the terms are my own] studied by Piéron ('09, and subsequent papers), who investigated the law according to which the sensitivity of snails to rhythmic shading was abolished. Piéron was, I believe, dealing with a condition primarily of sensory exhaustion. Both 'kinds of memory' are capable of mechanistic analysis, but that exhibited by Holothuria is more advantageous for experimental work. I venture to predict that the study of the impression of short-period rhythms upon animals, rather than the investigation of 'tidal memory' (of convoluta, etc., cf. Kafka, '14, Chap. 8), will afford the clue to the dynamics of primitive associative memory.

The muscles concerned in pulsation of the amputated cloacal end have previously been enumerated. Experiments were carried out to determine the significance of each of these. The results may be briefly stated thus:

Cutting out the cloaca, so that only the dermo-muscular tube and sphincter remained, resulted in complete cessation of movement. Scraping away the radiating muscles and connective-tissue strands had the same effect. Cutting the radiating muscles on only one side gave preparations which pulsated at normal rates; but in these preparations the side of the cloaca and brim on which the muscles were still intact closed and opened before the other side, the rest of the cloaca lagging behind in such a way as to give the impression of being "dragged along" with the intact half. The anal brim when cut out by itself remained open in sea water and did not pulsate, though it reacted, by a single closure, to delicate mechanical stimulations and to small volumes of various stimulating solutions.

If preparations of this sort, i.e., isolated sphincters—pieces deprived of the cloaca—or posterior ends in which all the radiating muscles had been cut, were placed in solutions of unusual Na/Ca content, they did exhibit rhythmic movement for some minutes. These effects were secured in solutions of the following composition:

1) 95 cc. sea water + 5. cc. $\frac{N}{10}$ sodium citrate. Cloacae with the radiating muscles cut pulsated for about fifteen minutes, but irregularly; parts of the anal sphincter closed before others, so that the anus presented a ragged outline.

2) Van't Hoff solution without Ca. Effect the same as in (1), but less marked. In both solutions isolated sphincters pulsated for several minutes.

3) $\frac{M}{16}$ NaCl. Cloacal ends with the radiating muscles cut pulsated slowly, but the sphincter did not open at all.

4) 100 cc. $\frac{M}{16}$ NaCl + 2 cc. $\frac{N}{10}$ NH_4OH. Slow pulsations, with incomplete closure of the anus.

In none of these tests did the pulsation last for more than a few minutes. These experiments are not, of course, decisive as regards an answer to the question of the relation of Ca to pulsation. Continuous stimulation resulting from the action of an exciting solution upon ectodermal sense organs, or upon muscles, might, in connection with the refractory period, produce the same result.

The integumentary nerve-net does not play a *necessary* part in the transmission of the wave of pulsation, since cloacal pieces having the integument cut in various ways (figs. 17 and 18) pulsated with complete coördination after some preliminary readjustment following the disturbances of the operation. To give an example:

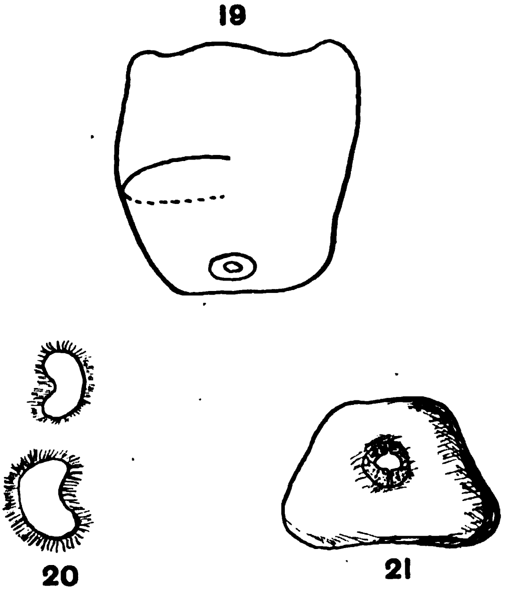
Experiment 84.2. July 22, 3.44 p.m. a cloacal piece, pulsating in sea water at the rate of 105 seconds for 10 pulsations, was cut as shown in figure 18, so that two separate rings of the integument were each connected with corresponding parts of the cloaca by the radiating muscles.

3.52 Posterior part pulsating feebly.
3.57 Both parts beating. Pulsations of normal amplitude; rate, 96.9 seconds for 10 movements.
4.10 103.9 seconds for 10 movements.
4.30 115 seconds for 10 movements.
8.45 Pulsating in irregular fashion.

The cloaca itself is therefore able to control the coördination of the pulsating system.

That the integumentary part of the apparatus does normally enter into transmission is indicated by tests made under the following conditions: 1) with the skin completely cut through on one side (fig. 19), and 2) with cloacal sphincter cut into lateral halves (fig. 20).

In the first case the sphincter and cloaca on the cut side lagged behind the opposite side, both in systole and in diastole.



Figs. 19, 20, 21 For explanation see text

In the second instance the brim no longer pulsated in coördinated manner and ceased to move after a few minutes. If one of the halves was gently pinched, it reacted alone, by the constriction of its circular muscles; whereas if stimulated more vigorously, or if a nearby point on the skin was stimulated, both halves of the brim contracted, the one nearer to the irritated place contracting sooner.

The effects of stimulation are thus conducted in a radiating fashion, such as would result if an ectodermal nerve-net were operating, and I conclude that such a net is present.

The rôle of the cloaca in pulsation, and especially of its anterior end, could be demonsrated very clearly. Isolated cloacal ends immersed in sea water containing $\frac{M}{200}$ caffeine presented this condition: During the early stages of the history of a piece in such a solution the anal sphincter pulsated at a normal rate of 10 movements in 70 to 80 seconds, but the cloaca was tightly contracted excepting immediately in the region of the anus (fig. 21). If now in such a preparation the contracted anterior end of the cloaca was exercised, all rhythm very promptly ceased. This points to the conclusion that the stimulus to pulsation originates at the anterior end of the cloaca with its associated radial muscles and is conducted posteriorly along the cloaca even when the cloaca itself is not pulsating. The fact that in certain salt solutions (e.g., NaCl M 5/8) the cloaca continued to beat after the anus had ceased, serves to strengthen this idea. The excised cloaca alone in sea water pulsated, though in a somewhat irregular manner. In recovery from the immediate effects of amputation, the cloaca and anal sphincter, and especially the former, began to pulsate before the body-wall portion of the complex.

It is taken for granted in this discussion that the coördinating mechanism is essentially nervous in character, and this view is supported by the experimental results. It has been suggested, however, that there is a chemical basis of coördinated pulsation in the case of the vertebrate intestine; Weiland ('12) claims to have extracted from the mammalian digestive tube a substance which, acting on Auerbach's plexus, leads to coördinated rhythmic movement. It must be admitted that the analysis of peristalsis upon the basis of the tonus idea (cf. Cannon, '11) does not account for the inception of the stimulus to contraction, and that therefore some further link is needed in the chain of pulsation processes.

d) Summary. The course of events in a pulsation cycle may be pictured as beginning with the opening of the anterior end of the cloaca, due to the relaxation of its circular muscles and the contraction of the associated radiating fibers. The stimulus derived from their contraction is transmitted posteriorly[10] by the integumentary nerve-net, while the wave of opening travels posteriorly along the cloaca, until finally the anal sphincter opens. Upon the contraction of the sphincter a wave of constriction travels anteriorly on the cloaca, forcing out the contained water.

IV. RELATION OF PULSATION TO TEMPERATURE

The large size of Stichopus precluded any attempt to employ the entire animal in a series of temperature experiments, both because there were no large thermostats available and because the thickness of its integument is prejudicial to the rapid establishment of uniform temperature conditions throughout the animal. For these tests therefore I employed Holothuria surinamensis and H. captiva; they were subjected to temperature changes in beakers of thin glass contained in a heating or cooling bath. Temperatures were measured by an enclosed-scale thermometer reading to 0.01°, placed close to the cloacal end of the animal. The instrument was calibrated. The results of one experiment, typical of all others, are given in figure 22.

This curve is entirely characteristic of the temperature curves found in connection with many other biological phenomena, in that it is of an exponential character, with a temperature coefficient (12.5°–22.5°) of about 2.4 ±.

In attempting to obtain temperature coefficients of pulsation rate in the case of the amputated cloacal ends of Stichopus, there entered a very considerable time factor. The method of procedure consisted in (1) obtaining records of rhythm in pieces which had recovered from operative shock and were subjected to fairly slow temperature changes (table 6), and (2) in subjecting similar pieces to sudden changes of temperature and estimating rhythm-rate after 5 to 10 minutes had passed and thermal equilibrium had presumably been established (table 7). The time factor enters in two ways, for in addition to the slow exhaustion which occurs even at the normal temperature and is itself probably affected by temperature, extreme thermal conditions also exercised a characteristic effect of their own which was manifested in a kind of hysteresis. By a condition of hysteresis I mean that subjecting a holothurian or its isolated cloacal end to

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[10] Experiments on Stichopus confirm the results obtained with H. surinamensis (Crozier, '15 [?]), to the effect that the responses to sensory stimuli in the posterior region of the animal tend to be conducted posteriorly toward the anus, which often reacts before there is visible any local response to the stimulating agent.

TABLE 6

Experiment 33.2. June 21. Stichopus moebii, 31 cms. long; 83 seconds for ten pulsations

TIME	MINUTES	TEMPERATURE	SEC./10 P.	NOTES
		Deg. C.		
8.40				Cut off end.
8.46	6	24.5	80.8	
9.04	14	25.0	122.0	
9.06	16	26.5	118.0	
9.07	17	27.6	105.0	
9.08	18	28.7	98.0	
9.10	20	29.4	100.0	
9.11	21	29.7	135.0	
9.12	22	29.8	143.0	
9.15	25	30.0	150.0	Open phase prolonged.
9.19	29	32.0	164.0	Parts dissociated.
9.25	34	34.0	∞	No movement.

TABLE 7

Experiment 39.4. June 24. Stichopus moebii 29 cms. long; 74 seconds for 10 pulsations, at 24.3°. Fifteen minutes after excision, plunged successively into water at different temperatures

TEMPERATURE	TIME FOR TEN PULSATIONS
Deg. C.	*seconds*
25.0	94
14.0	198
16.0	187
17.0	186
19.5	140
21.2	127
19.6	144
20.0	155
15.0	192

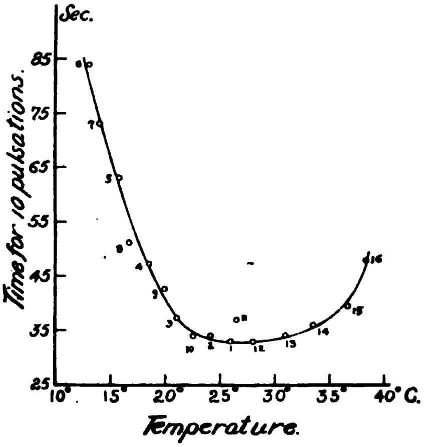

Fig. 22 Holothuria captiva. Relation of pulsation rate, in the intact animal, to temperature. The numbers on the curve indicate the succession in which the observations were taken.

a temperature of, say, 10°, produced an anaesthetised-like condition which persisted for a considerable time after its removal to some higher temperature and thermal equilibrium had in all probability been reached. By way of illustration:

Experiment 39.2. June 24. *Stichopus moebii,* 27 cms. long; 78 seconds for 10 pulsations at 25.0°; slowly cooled.

TIME	MINUTES ELAPSED	TEMPERATURE	TIME FOR TEN PULSATIONS	NOTES
P.M.		*Deg. C.*	*seconds*	
3.25	0	25.0	64	Six minutes after amputation
3.33	8	25.0	88	
3.37	12	22.0	109	
3.45	20	12.0	215	
4.00	35	10.0	∞	Closed; no pulsation
4.20	55	17.0	∞	Beginning to open slightly
4.30	65	18.2	∞	Opening slowly
4.45	80	18.5	216	Not opening completely
4.50	85	18.9	188	Fuller amplitude

Experiment 34.1 June 21. *Stichopus moebii,* 29 cms. long; 76 seconds for 10 pulsations at 25.0°; cloaca amputated; during the following 18 minutes, slowly heated up to 30°; held at 30° for 10 minutes, with the following results.

ELAPSED TIME	TIME REQUIRED FOR TEN PULSATIONS	NOTES
minutes	*seconds*	
2	135	
5	146	
8	198	
10	∞	Wide open; no pulsation

Then cooled down slowly (for 19 minutes) to 20.0°; pulsations (slowly revived) required 150 seconds for 10 movements (the usual rate 60 minutes after amputation being 60 to 75 seconds for 10 pulsations). All movements ceased in about 10 minutes more.

These experiments were checked by many others of similar type which gave essentially the same result.

The main object of these tests being to ascertain the magnitude of complications possibly introduced into the experiments by temperature effects, there is given in figure 23 some of the curves derived from different tests under several of the possible conditions of temperature change, and in figure 24 are collected the data from all the temperature experiments with isolated cloacae.

The discussion of these results with regard to the significance of the temperature coefficient would be unprofitable,[11] since (in view of the source of complication above noted) decisive evidence could be obtained only by the study of isothermal exhaustion curves. But it may be pointed out that the hysteresis effect to which attention has been called indicates that some physical alteration of the substance of the pulsating tissues has occurred at the temperatures which produce this hysteresis effect. It is difficult to believe that this influence is discontinuous, and, as Adrian ('14) has most recently pointed out, the van't Hoff equation holds only in homogeneous systems in which no alteration of the components is induced by temperature changes. In other words, there is a time-factor to be considered when working with temperatures removed from the normal. It is the neglect of this was by this means (as well as by the movements of the body wall) efficiently stirred as long as the piece continued to pulsate; local diffusion changes were thus avoided. One disadvantage of this whole method lies in the fact that nothing could be done to prevent the action of the solutions upon the cut surfaces along the plane of amputation. The magnitude of this effect could not be estimated.

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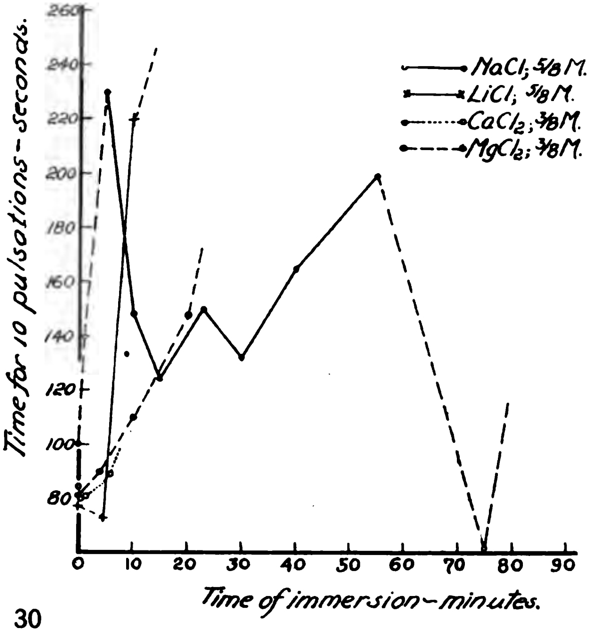
[11] Pütter ('14) has recently given an exhaustive treatment of the temperature-variability curve from the standpoint of the linked reactions occurring in living systems.



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Individual records illustrating the action of the single salt solutions in which cloacal pulsation occurred are plotted in figure 30. Table 9 contains a summary of the results of these experiments. With NH_4Cl and KCl, although the anal sphincters were tightly closed, some irregular pulsation of the body wall usually was evident for about the length of time indicated in table 9.

The order of the disappearance of tactile irritability parallels that of the stoppage of pulsation in these salt solutions. The completeness of the correspondence argues for a close similarity in the mechanisms of stimulation by internally and externally generated agencies.

washed (inside and out) with a stream of fresh water[16] before being put in the non-electrolyte solution. No evidence was had of an increase in muscular tone in sugar solutions, though pulsation usually ceased with the sphincter in the contracted condition. I had previously found (Crozier '15) that glycerin and maltose would stimulate the skin of Holothuria surinamensis in a sensory way; this effect probably had nothing to do with the action of sugar solutions upon pulsation, since the glycerin and maltose solutions did not behave differently from those of sucrose, etc., which had been found not to stimulate. Pulsations, where they did occur, were always of lower frequency in sugar solutions than in the controls in sea water.

VIII. ION EFFECTS

a) *Single salts*. The relation of the salts of sea water to cloacal pulsation and general irritability was studied by observing the action of single electrolytes at equivalent concentrations (i.e., isotonic with sea water), and the effects of various combinations of these substances in the proportions at which they occur in sea water. The method of procedure consisted in placing in the desired salt solution freshly amputated cloacal ends of which the pulsation rate had been determined previous to removal from the animal. Before immersion the pieces were rapidly washed by a stream of rain water from a wash bottle. Check experiments showed that washing the outside and inside of the cloacal end in this way did not have any effect upon the rate or duration of subsequent pulsation of pieces reimmersed in sea water. Since the excised pieces were immersed while in the condition shown in *A*, figure 12, that is, before the inturning of the cut edges had closed the cavity containing the radiating muscle strands, the solution had abundant opportunity to gain access to the elements concerned in pulsation. Care was taken that the cavity became filled with the solution surrounding the immersed piece and that no air remained in it. Since the pumping movements maintained a current of fluid through the cloaca, the solution of the sea water (down to 80 per cent of the original concentration), whereas the rate of pulsation in intact medusae (p. 28) is not increased in this way; but in his tables no records are given of the pulsations of entire medusae in sea water of the critical concentrations (95 per cent, 90 per cent). It might appear from the records in my figure 28 that there is some tendency on the part of diluted sea water to preserve in the excised cloacal ends of Stichopus a higher rate of pulsation during the later stages of their history, than is the case with the 'control' pieces in 100 per cent sea water. But, as a matter of fact, this is not so, since there was enough variation in these records and in the controls to prohibit a conclusion of this sort. The matter was studied more carefully with 90 per cent and 95 per cent sea water; no increase in pulsation frequency was discoverable upon diluting to these concentrations sea water in which amputated cloacal ends were pulsating.

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[16] Check experiments showed that the washing had no effect on pulsation, at least on its duration.

b) *Non-electrolyte solutions.* It was found by Loeb ('00 [a]) that in solutions of non-conductors the isolated center of Gonionemus did not exhibit rhythmic contractions. This has in general been the experience of others employing a variety of pulsating mechanisms, namely, that in a medium free from salts but with its osmotic pressure made·up to normal by dissolved non-electrolytes, rhythmic contractions are either not initiated or do not continue for any length of time. According to Mayer ('14 [b]) this physiological inefficiency, of sugars and the like, is also apparent in nerve-conduction in Cassiopea.

The results of experiments in which the isolated cloacal end of Stichopus was placed in solutions of non-electrolytes may be given very briefly. Pieces immersed in solutions of sucrose, lactose, maltose, or glycerin theoretically isosmotic with sea water (i.e., 0.9 to 1.0 M) did not pulsate for more than about 15 minutes, the actual time for the duration of pulsation varying from 2 to 25 minutes. Irritability to mechanical stimulation disappeared after about half an hour. Such pieces did not recover on return to sea water. In these tests the amputated cloacal end was allowed to begin its pulsation in sea water and to continue there for about 10 minutes; the pieces were then and the various dilutions of sea water, namely that in the former case the sphincters remained wide open, whereas in the latter instances the sphincters (as in 'normal' exhaustion in 100 per cent sea water) were more or less contracted, though with the rain-water effect it was not a matter of the contraction of the anal dilators, but rather of the more complete relaxation of the circular constrictors.

With rain water it was found that the brownish skin pigment rapidly made its appearance in the water surrounding a beating cloacal end even before pulsation ceased. This effect became apparent after immersion of about 5 minutes. It was, under certain circumstances, found with other concentrations of sea water, but (as will be shown subsequently) it could be inhibited by the presence of non-electrolytes. This reaction may indicate either that the permeability of the superficial cells had been very highly modified, or that the cells surrendering their coloring matter were dead; inasmuch as the pigment loss did not occur more at the region of the cut end of the pieces than at any other place, and could be inhibited by the addition of sea water, and further inasmuch as the region of the cut end did not give off any visible amount of pigment into normal sea water, it might reasonably be held that the latter alternative is not necessarily the correct one. It is possible that at concentrations much removed from that of normal sea water the cells of Stichopus became rather highly permeable for salts while still alive. This possibility was suggested by the very evident amounts of chlorine found in rain water in which pulsating pieces had lain. If this permeability for salts could be proved over the lower range of concentrations here dealt with, the form of the lower parts of the curves in figure 29 might readily be accounted for; between 0 per cent and 95 per cent these graphs might result if the critical internal conditions (salt concentrations?), beyond which no spontaneous pulsation is possible and irritability ceases, were approached (1) by the intake of water and at the same time (2) by the exit of salts.

It is stated by Mayer ('14 ^{b}, p. 40) that the rate of nerve conduction in operated Cassiopea is accelerated by slight dilution to be immersed in a given volume of diluted or evaporated sea water were first washed (30 to 60 seconds) in a stream of sea water of the corresponding concentration. The volume of solution containing a single isolated cloacal end was in each case 300 cc.

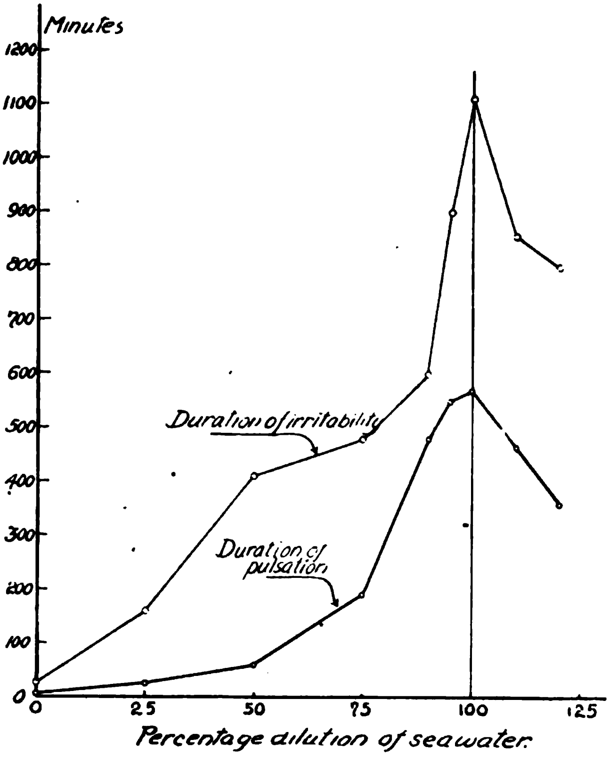
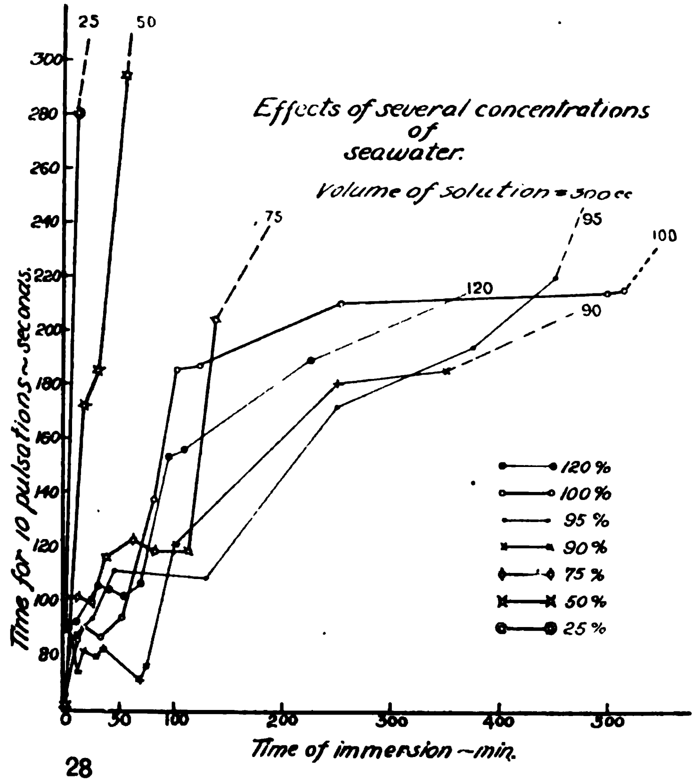


Fig. 29 Based on experiments with 45 specimens of Stichopus

Five individual pieces were carefully studied at each concentration of sea water and their behavior checked by less detailed observations upon a number of other examples. The average times for the continuation of pulsation and of irritability to mechanical stimulation under the conditions just specified (at 25° to 27°) are given graphically in figure 29. As regards the condition of the cloacal sphincter after the cessation of pulsation, this difference was noted between the action of rain water vidual cases is plotted in figure 28. The tests indicated that above 50 per cent concentration, mixtures of sea water with rain water tended to preserve about the normal pulsation-rate, but that in these mixtures the duration of pulsation was less than that of the control (100 per cent); below 50 per cent sea water



28

the pulsation-rate and duration fell off rapidly. Above 100 per cent concentration the duration of pulsation was also curtailed. The general nature of these effects did not differ whether (a) the cloacal pieces were immersed in the solution immediately after amputation, or (b) after pulsation in the amputated end had been allowed to start in sea water; procedure 'a' was followed in the cases recorded.[15] In these experiments the pieces

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[15] With this method of immersion, the pieces were in the condition shown at *A* in figure 12, and the solution therefore had free access to the interior parts of the cloacal end.

The cloacae exposed to light did not recover on return to the shade. Light exerted its typical effect upon the excised cloacal ends, and at a rate which is comparable to that at which it causes the disappearance of sensitivity to shading in the intact animal (Crozier '14, '15).

e) *Summary.* Lack of oxygen would appear to be much less powerful as a depressant of rhythmic activity than the presence of urea,[12] CO_2, and other typical metabolic products (the effects of acids will be discussed subsequently in a separate section), though the suppression of oxidations by KCN is rapidly effective in stopping movement. In those cases in which light produces toxic modifications in the animal (H. surinamensis, H. captiva), this agent also causes rapid cessation of rhythm.

VII. INFLUENCE OF OSMOTIC PRESSURE

a) *Dilutions of sea water.* According to Henri et Lalou ('03), the membranes of Stichopus regalis which are exposed to contact with water are almost perfectly semi-permeable, the salt concentration in the ambulacral[13] and perivisceral fluids being normally slightly less than that in the sea water. They found that S. regalis readily accommodated itself to moderate dilutions of the sea water in which it was placed.

In the experiments about to be described it was found that diluted and concentrated sea water exerted characteristic effects upon cloacal pulsation. It should be remembered that in these experiments with the isolated cloacal ends the medium had access to the internal surfaces of the body wall and cloaca, and further that the cut surfaces of the integument and cloaca at the exposed end were freely open to its action.

The pulsation-rate of amputated cloacal ends was observed in sea water at the following volume percentage concentrations: 0, 25, 50, 75, 90, 95, 100, 110, 120.[14] The history of typical indiof the isolated cloacal ends. Stichopus is much less sensitive to photic irritation than is Holothuria, and no influence of light upon either rate or amplitude of pulsation could be detected. In Holothuria it has been shown with some degree of probability that the green fluorescent integumentary pigment acts as a photosensitizer (Crozier, '14); hence a photic effect upon cloacal pulsation would be much more probable in this case.

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[12] According to Henri et Lalou ('03) the membranes of Stichopus regalis are impermeable to urea, but in my experiments a cut surface was exposed to the action of the fluid. Furthermore, the remnant of the body cavity was freely open.

[13] From the Polian vesicle.

[14] The dilutions from 100 per cent were made by the addition of rainwater; the 110 per cent and 120 per cent solutions were made by evaporation of sea water to the required volume. Normal Bermuda sea water contains about 36.8 p.p.m. salt (Mark, '13).

Isolated cloacal extremities of Stichopus which had ceased to pulsate regularly in sea water, though otherwise in apparently good condition, were placed in moderately bright sunlight. This did not stimulate to pulsations. When such pieces were shaded, the sphincter reacted by closing fairly tightly; the reaction time was about 1.2 seconds at 25.0°. Pieces which were pulsating slowly (130 seconds ± for 10 movements) gave the same reaction, the sphincter closing promptly when the shadow was cast during the open phase, but failing to react during a period of closure. Light did not accelerate the rate of movement in normally pulsating cloacal ends.

The isolated cloacal extremity of H. surinamensis, however, gave the following result:

Experiment 53.2. Seven specimens of *Holothuria surinamensis* had the posterior ends (1.5 cms. ±) removed. After remaining about 30 minutes in diffuse daylight, their pulsation rates were observed (column *a*). Four of the pieces were then placed in bright sunlight (*b′*), the other three remaining in the diffuse daylight (*b*). After 15 minutes the pulsation rates were again determined (*b*, *b′*):

Time for ten pulsations, seconds

IN DIFFUSE LIGHT		AFTER FIFTEEN MINUTES IN BRIGHT SUNLIGHT	
a	*b*	*b′*	Notes
80		137	Very irregular
90		54	Brim only beating
84		∞	
130		∞	
70	72		
81	80		
76	84		

This effect was uniformly obtained in other experiments of similar nature.

b) *Carbon dioxide.* Carbon dioxide was a powerful agent in suppressing pulsations. Tests were made by adding to sea water small volumes of rain water charged with CO_2. The carbonated water was slightly acid aside from its H_2CO_3.

Experiment 79.2. July 11. Three freshly amputated cloacal ends were immersed each in 200 cc. of sea water to which 2 cc. of 'carbonated water' had been added. They did not pulsate in this mixture. After ten minutes immersion the pieces were transferred to normal sea water. Pulsations revived in 5 to 7 minutes.

Concordant results were obtained by adding CO_2-water to solutions containing pulsating pieces, the pulsations being rapidly reduced in amplitude and usually stopped within a few minutes when from 1 to 2 per cent of the 'carbonated water' had been added.

c) *Urea.* Urea likewise had a depressing effect upon pulsation. Fosse ('13) identified urea in echinoderms and their excretory products.

Experiment 38.2. June 24. *Stichopus moebii,* 27 cms. long; 68 seconds for 10 pulsations at 25.1°; cloacal end amputated and placed in 300 cc. sea water + 5 cc. $\frac{M}{10}$ urea.

IMMERSION	FOR TEN PULSATIONS	NOTES
minutes	*seconds*	
153	151	Very long open phase
184	170	Irregular amplitude
214	103	Somewhat improved
242	164	Open phase long
360	∞	Sphincter half open; reacts only slowly to tactile irritation

This particular experiment is plotted at *C* in figure 27; it was checked by four other experiments, which yielded the same result.

d) *Light.* With Holothuria surinamensis and H. captiva it was previously found (Crozier, '14, '15) that light exerted a distinctly toxic influence on the animals. It was therefore expected that sunlight would affect in some way the pulsation rate

Experiment 71.2. July 25. *Stichopus moebii* 27 cms. long; cloacal end amputated and immersed in 200 cc. sea water + 5 drops $\frac{M}{10}$ KCN.

TIME OF IMMERSION	TIME FOR TEN PULSATIONS	NOTES
minutes	*seconds*	
5	80	Not closing completely
10	147	Closed phase very brief
17	84	Full amplitude
23	111	Closure not complete
38	∞	Irregular movements
53		Dead

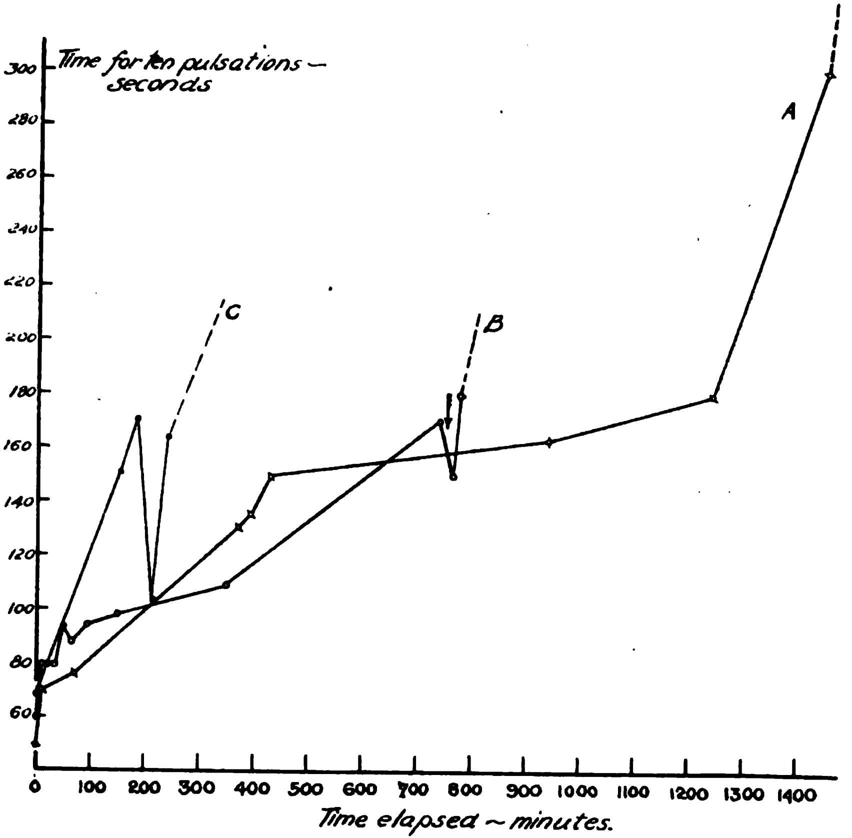


Fig. 27 *A* = control, in sea water. *B* = in sealed jar of boiled sea water. *C* = sea water (300 cc.) + 5 per cent urea (5 cc.).

Note: At the point marked ↓, 'B' had ceased to pulsate, and was transferred to normal sea water, in which it temporarily revived.

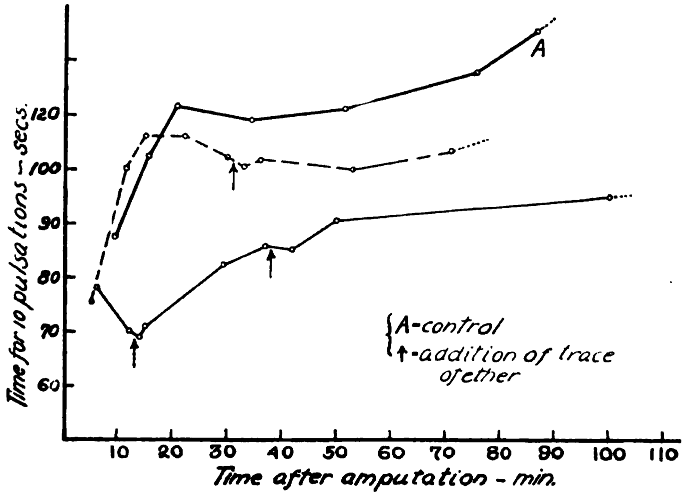
VI. RELATION OF PULSATION TO OXYGEN AND METABOLIC PRODUCTS

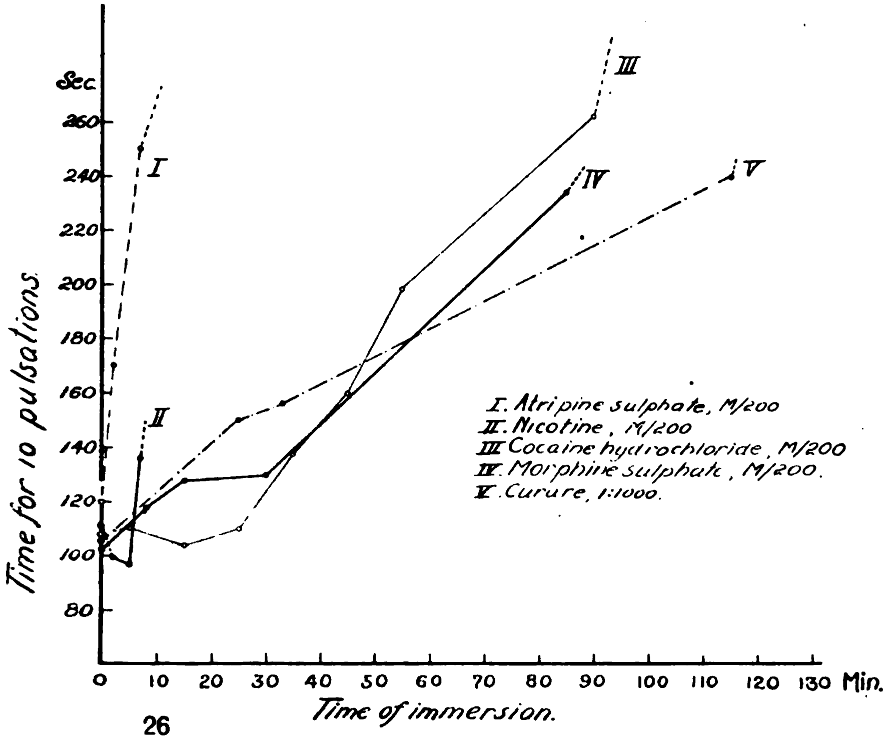
a) *Oxygen.* It has previously been shown that holothurians would live for relatively long periods in sealed jars of boiled sea water (Crozier '15). This was in conformity with the observations of Moore and his colaborators ('12, '14), showing the small amounts of dissolved oxygen used up in the activity of some marine animals, including echinoderms. The isolated cloacal ends of Stichopus continued to pulsate for over 700 minutes in jars of sea water which had been boiled, sealed and subsequently cooled to room temperature out of contact with the air. In preparing this water the evaporation was very slight, but possibly some faint increase in alkalinity was induced. The course of a typical experiment is illustrated in figure 27 (*B*), plotted from the record of experiment 78.3, with which may be compared the control (*A*), begun at the same time.

Experiment 78.3. July 10–11. *Stichopus moebii*, 20 cms. long; 60 seconds for 10 pulsations at 23.5°; volume of boiled sea water, 550 cc.

MINUTES ELAPSED	TIME FOR TEN PULSATIONS	NOTES
	seconds	
0		Cloacal end amputated and put in sealed jar of boiled sea water
10	78	
20	78	
35	78	
50	91	
65	88	
95	92	
350	109	Amplitude decreasing
740	170	Pulsations feeble and irregular
752		Taken out of jar and transferred to fresh sea water. Not pulsating
764	150	Closure nearly complete but more or less irregular
778	180	Irregular movements; no wide-open phase
800		Twitching irregularly; barely alive

The dependence of pulsation upon oxidations is probably indicated, however, by the high toxicity of KCN, as, for example, in


Time for 10 pulsations – secs.
60
70
80
90
100
120
10
20
30
40
50
60
70
80
90
100
110
Time after amputation - min.
A
A-control
↑-addition of trace of ether


Time for 10 pulsations.
Sec
80
100
120
140
160
180
200
220
240
260
0
10
20
30
40
50
60
70
80
90
100
110
120
130 Min.
Time of immersion.
I
II
III
IV
V
I. Atripine sulphate, M/200
II. Nicotine, M/200
III Cocaine hydrochloride, M/200
IV. Morphine sulphate, M/200.
V. Curare, 1:1000.

In all cases of anaesthetisation the quiescent condition showed the anal sphincter in diastole and the cloaca itself more or less closed, that is, with the radiating muscles relaxed.

Traces of anaesthetics commonly induce a temporary acceleration in the rate of rhythmic movements. This is also the case with the cloaca of Stichopus (fig. 25). The effect on pulsation *rate* was less evident than the concomitant increase in amplitude and vigor of contraction. The effect lasted only a few minutes.

TABLE 8

Volume of solution in each case = 250 cc.

SUBSTANCE	CONCENTRATION	DURATION, IN MINUTES, OF		NOTES: CONDITION OF ANAL SPHINCTER AFTER STOPPAGE
		Pulsation	Irritability	
Curare	1 : 1000	130	170	Circular body muscles contracted, longitudinal ones relaxed; = open
Caffeine	M/200	25	160	Cloaca contracted; sphincter open
Nicotine	M/200	8	12	Contracted
Cocaine hydrochloride	M/200	90	100	All muscles relaxed
Atropine sulphate	M/200	28	60	Wide open; radiating muscles strongly contracted
Morphine sulphate	M/200	140	165	Relaxed
Sea water control	....	540±	1100±	Sphincter ± open, all muscles relaxed

b) *Alkaloids.* The influence of depressing alkaloids was also studied; in each instance the history of five individual isolated cloacal ends being followed in detail and the general result checked by less detailed observations upon other pieces. The results with regard to pulsation and irritability to mechanical stimulation are summarised in table 8. Representative individual curves of exhaustion in these solutions are plotted in figure 26.

The powerful effects of nicotine and atropine are comparable to their influence on many other types of smooth muscle.

It is interesting that the temperature of maximum pulsation rate should be so near the temperature, at this time of year, of the sea water in which the animals were living; this was 24° to 27° in the day time.

V. ACTION OF DEPRESSING AGENTS

a) *Anaesthetics.* The effects of typical anaesthetics upon rhythm in the isolated cloaca of Stichopus are illustrated by the following:

Chloroform

Experiment 46.1. July 29. *Stichopus moebii,* 21.5 cms. long; before excision of cloaca, 61 seconds for 10 pulsations.

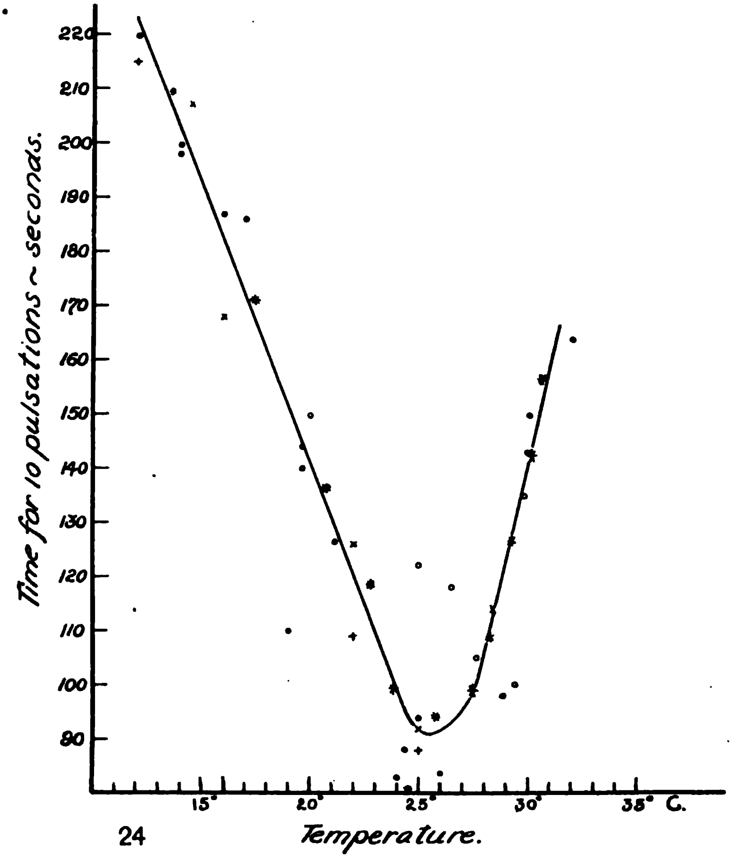
TIME ELAPSED AFTER AMPUTATION	NOTES	TIME FOR TEN PULSATIONS
minutes		*seconds*
3	Began to pulsate in sea water	
11		56
15	Transferred[2] to sea water half-saturated with $CHCl_3$	
20		113
21		120
22	Pulsation stopped	∞
24	Washed, and put in normal sea water	∞
40	Recovering amplitude	105
57	Amplitude fully recovered	130
90	Normal	145

---

[2] Previous tests had shown that transferring the pieces from one solution to another had no effect on pulsation rate, even though during handling the sphincter was closed.

Ether, ethyl alcohol and ethyl carbamate gave similar results, i.e., the depression of rhythm was, within limits, reversible. Magnesium sulphate to the extent of 0.25 M added to sea water stopped pulsation in about 10 to 12 minutes. Urethane and chloretone were compared quantitatively, with the result that when freshly isolated cloacal pieces were immersed in $\frac{M}{200}$ chloretone in sea water they ceased to pulsate in less than 3 minutes, whereas in $\frac{M}{200}$ urethane pulsation lasted for as much as 80 minutes.

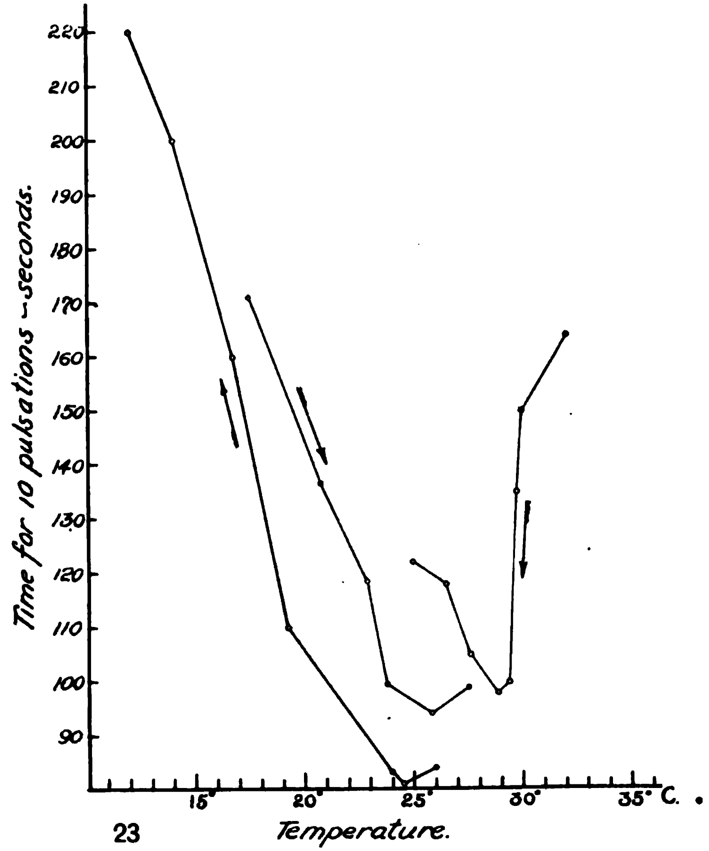
limit to the physiological temperature scale than there is a lower one (Cameron and Brownlee, '13). On either side of the minimum in "time for 10 pulsations" the curves are rather steep and of exponential character, though this is obscured in the collected



24

data (fig. 24). The range of laboratory temperatures, obtained from temperature measurements in connection with each experiment, was from 24° to 27°, hence very little, if any, complication due to a temperature effect entered into the subsequent experiments.

factor which renders valueless for exact purposes much of the experimental evidence concerning the influence of temperature upon marine animals (e.g., as in the paper of Mayer, '14 [a]); it is simply incorrect to say that an animal dies at such and such a temperature, for it dies at T° after being heated thereto at a certain rate and kept at T° for a certain length of time.



23

The curves (figs. 23 and 24) show that there is a rather sharp maximum of pulsation rate in the neighborhood of 26°. No pulsation was apparent below 12.5°, nor above 32.0°. These limiting temperatures hold for the rates of heat change, etc., used in these experiments; there is no more a definite upper

TABLE 9

Effects of single salts

SALT	M. CONCENTRATION	DURATION OF	
		Pulsation	Irritability
		minutes	*minutes*
NaCl	5/8	75	105
LiCl	5/8	12	15
NH_4Cl	5/8	(10) 0	12
KCl	5/8	(3) 0	10
$CaCl_2$	3/8	5	7
$MgCl_2$	3/8	20	30
$MgSO_4$	9/10	17	24

Averages from five series of tests. The figures in parenthesis opposite NH_4Cl and KCl mean that, while the sphincter of cloacal pieces immersed in these solutions did not pulsate, the body part did so, irregularly, for the number of minutes noted.

TABLE 10

Summarizing the results of tests made upon five isolated cloacal ends of Stichopus moebii with each of the salts indicated

SALT	NaCl		LiCl		NH_4Cl		KCl	
Concentr., molecular	5/8	5/16	5/8	5/16	5/8 (10)	5/16	5/8 (3)	5/16
Duration of pulsation	75	50	12	35	0	0	0	0
Duration of irritability	105	80	15	38	12	41	10	15

Time—Minutes.

For the chlorides of the alkali metals and radical (table 10), the order

$$Na > Li > NH_4 > K$$

was obtained with reference to their ability to preserve pulsation and irritability. The significance of this series, in terms of the aggregation state of protoplasmic materials, has been treated in a comprehensive manner by Höber ('14, pp. 471 et seq.). A beautiful demonstration of the action of the cations of neutral salts in this series has recently been given by Spaeth ('13) for the chromatophores of Fundulus. In solutions of NaCl and LiCl the pieces came to rest with the anal sphincter in an expanded condition but with the radiating cloacal muscles contracted. This was unlike the condition in solutions of NH_4Cl and KCl, where both cloaca and anal sphincter were tightly contracted. The absence of any pulsation in 5/8 M NH_4Cl and in 5/8 M KCl is partly a secondary result, since solutions of these salts are powerful sensory stimulants for the skin of holothurians (Crozier, '15), which produced contractions from which the cloacal ends did not recover before the toxic action of the solutions led to death.

That the single electrolytes LiCl, NH_4Cl, and KCl are *toxic*, is also indicated by the results of tests in which several concentrations of these substances were compared (table 10). These experiments showed that with LiCl the duration of rhythmic movement and of irritability was greater at 5/16 M than at 5/8 M concentration, and that with NH_4Cl and KCl the duration of irritability was also greater at the lower concentration, even though in the case of the more dilute solutions an osmotic factor (see previous section) was also working to produce death.

NaCl is thus the only single constituent of sea water which will maintain pulsation and irritability for any considerable length of time, and in this respect it is only partially imitated by LiCl —a fact further proven by the physiological incompleteness of a van't Hoff solution made up with LiCl in place of NaCl, in which the pulsation of a cloacal end endured for about 45 minutes.

The comparative effects of $MgCl_2$ and $MgSO_4$ were of particular interest. Spaeth ('13, p. 553) found that $MgSO_4$ exerted a more powerful action upon the melanophores of Fundulus than did an iso-ionic $MgCl_2$ solution. Table 11 contains the data from experiments in which $MgCl_2$ and $MgSO_4$ were compared at several concentrations by means of their action on cloacal pulsation in Stichopus. The result of these tests was that $MgSO_4$ appeared to have a distinctly higher anaesthetic power than $MgCl_2$.

b) Mixed salts. As the basis for the experiments made with mixed salts there was employed a van't Hoff solution of the composition: 5/8 M [100 NaCl + 7.8 M_gCl_2 + 3.8 $MgSO_4$ + 2.2 KCl + 2.5 $CaCl_2$] (cf. Mayer, '11, '14 [b]). The solutions were made up in rain water. The averages obtained for the time of

TABLE 11

SALT	$MgCl_2$		$MgSO_4$	
Concentrated, molecular	5/16	3/8	5/16	9/10
Duration of pulsation, minutes	14	20	14	17
Duration of irritability, minutes	37	30	26	24

duration (a) of pulsation and (b) of irritability to mechanical stimulation, in the several salt mixtures studied, are given in table 11. By way of comparison, data taken from Bethe's account of the effects of sea water constituents upon pulsation in medusae (Bethe, '08) are given in the last column of table 12. The cloacal ends of holothurians are in some ways a better indicator for use in studies of this nature than are medusae, since the former pulsate continuously and not, as the jelly fishes do, in more or less interrupted groups of movements.

TABLE 12

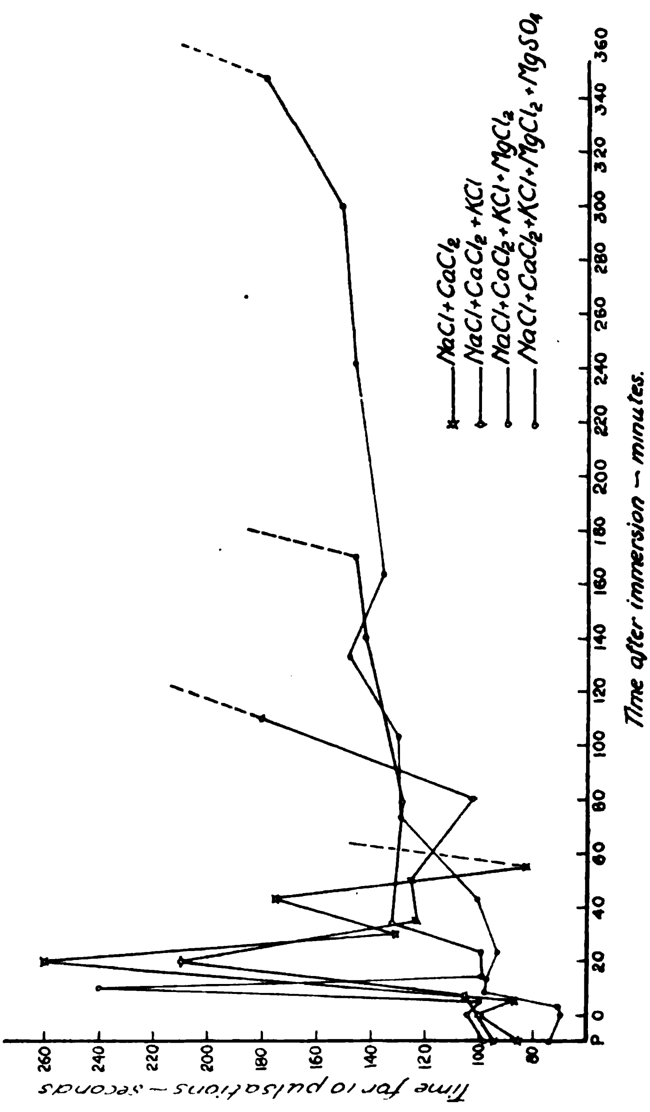
Summarizing the results of tests with salt mixtures. For details, see text

	SOLUTION	DURATION OF Pulsation	DURATION OF Irritability	R.
		minutes	*minutes*	
1	NaCl	75	100	27
2	NaCl + $CaCl_2$	60	280	95
3	NaCl + KCl	50	90	47
4	NaCl + KCl + $CaCl_2$	120	260	150*
5	NaCl + $MgCl_2$	42	60	1
6	NaCl + KCl + $CaCl_2$ + $MgCl_2$	104	300+	
7	van't Hoff solution	360	480	1200±
8	"7" made alkaline	600		
9	Sea water	540		(10 days)

In column 'R' are given the corresponding data for the pulsation of the medusa *Rhizostoma* (Bethe, '08, p. 573).

* This solution contained $CaCO_3$.

A notable peculiarity of the pulsation curves of pieces immersed in NaCl solution (compare previous section, and figure 30) is the irregularity of the pulsation rate. During the early history of pieces in NaCl, the pulsation rate was also very low. By reference to figure 31 it will be seen that, in the experiments


NaCl+CaCl$_2$
NaCl+CaCl$_2$+KCl
NaCl+CaCl$_2$+KCl+MgCl$_2$
NaCl+CaCl$_2$+KCl+MgCl$_2$+MgSO$_4$
Time after immersion — minutes
P
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
Time for 10 pulsations — seconds
80
100
120
140
160
180
200
220
240
260

plotted, only in the presence of $MgSO_4$ is the sodium effect completely abolished. This was quite uniformly observed. $MgSO_4$, in combination with NaCl, KCl, and $CaCl_2$, led to pulsations of a normal character, whereas $MgCl_2$ did not do so, not completely at least, even in mixtures where the quantity of Mg normally derived from $MgSO_4 + MgCl_2$ was "made up" by a calculated amount of $MgCl_2$. The deficiency of solutions lacking magnesium lay in the fact that, as Loeb ('06) observed with the medusa Polyorchis, in these mixtures the sphincters tended to contract permanently, in more or less tetanic fashion. $MgCl_2$, and especially $MgSO_4$, and more particularly both together, acting with the other salts of sea water, led to normal relaxations after each systole, and thus tended to preserve a normal rate and duration of pulsation. Solutions containing NaCl and $MgCl_2$ or $MgSO_4$, or both, did not maintain rhythmic movements as long as controls in NaCl; $CaCl_2$ and KCl were necessary for the complete balance of the solution, as regards pulsation.

This applies to artificial salt mixtures. They were practically neutral in reaction. An attempt was made to approach the problem from the other side, by precipitating the SO_4'' out of sea water by $BaCl_2$. Solutions prepared in this way preserved pulsation for not quite so long (about 4 hours) as did the neutral van't Hoff mixture. The effect noted in the absence of $MgSO_4$ in artificial salt combinations may therefore have been due, in part, to the particular $C_{H^\cdot}$ of those mixtures (cf. Loeb, '10).

It is unnecessary to go into the analysis of many of the results obtained with salt mixtures, since they are so similar to those which have been found for other pulsating structures (Robertson, '10). Certain findings with reference to the significance of $Ca^{\cdot\cdot}$ in pulsation may however be mentioned. From table 12 it will be seen that the antagonism between $Na^{\cdot\cdot}$ and $Ca^{\cdot\cdot}$ was a very imperfect one as regards the duration of pulsation, though good as determined by the preservation of irritability. Between $Na^{\cdot}$ and $K^{\cdot}$ there was no antagonism. $Na^{\cdot} + K^{\cdot} + Ca^{\cdot\cdot}$ formed a nearly completely balanced solution, aside from the $K^{\cdot}$ and $Ca^{\cdot\cdot}$ tetanising effects, which interfered with normal diastolic relaxation. The preservation of irritability was not very much less efficient in NaCl + KCl + $CaCl_2$ than in the same + $MgCl_2$. The depressant action of salts which diminish the active number of Ca ions has frequently been assumed to be due to their effect on the $Ca^{\cdot\cdot}$ concentration, but the results of Salant and Hecht ('15) indicate that this conclusion is not well founded. Experiments of the following type showed that these well known relations obtained also with the cloaca of Stichopus:

Experiment 45.2. June 29. Five preparations from *Stichopus moebii* were allowed to begin pulsating in sea water. They were then transferred to a 0.1 per cent solution of oxalic acid in sea water. Pulsations of the sphincter ceased in 3 minutes, though the cloaca and body wall contracted rhythmically for about 4 minutes longer. The sphincter remained open; it contracted once in response to each light touch, for 5 minutes more.

Experiment 75.2. July 20. Three cloacal preparations immersed in a solution of the following proportional composition: 100 cc. sea water + 3 cc. $\frac{N}{10}$ tartaric acid. Pulsation lasted 15 to 20 minutes. Ceased with the cloaca and sphincter in the *open* phase.

Experiment 74.2. July 20. Five cloacal preparations immersed in a solution of the following composition: 100 cc. sea water+ 5 cc. M 5/8 sodium citrate. Pulsations continued for a little over two hours, ceasing in the *open* phase. The movements, while they lasted, were of almost abnormal amplitude and vigor. Cessation found the sphincter in the open phase.[17]

Experiment 74.1. August 3. Three cloacal preparations immersed in a solution of the composition 100 cc. sea water + 2 cc. M 5/8 $CaCl_2$. Pulsations continued for about 5 hours, at the end of which time the sphincters were all tightly closed, though they responded to a touch by opening and closing once.

Further increase in the amount of $CaCl_2$ in sea water stopped pulsation (with the characteristic calcium tetanus) in a shorter time.

The efficiency of salt mixtures in preserving pulsation was notably improved by making them alkaline, especially with NH_4OH. NH_4Cl was found to be very toxic, but solutions of the composition: 100 cc. sea water + 2 cc. $\frac{N}{10}$ NH_4 OH preserved pulsation for about 20 hours, when the volume of solution for each piece was 500 cc. NaOH or KOH substituted for the

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[17] In this solution the tube feet moved incessantly, in sharp contrast to their usual quiescent condition in sea water.

NH_4OH did this for about 15 hours. The amplitude of pulsation in these mixtures was greater than in controls run in sea water. It is suggested that alkalis favored the continuance of rhythm by assisting in ionic exchanges at the surface of the contractile elements; probably this was accomplished in part in a secondary way, namely, by the neutralization of acid metabolic products.

c) *Hydrogen-ion concentration.* The hydrogen-ion concentration of sea water is given by various authors as lying between 0.5×10^{-8} and 1.5×10^{-5} (Höber, '14, p. 195). The sea water used in experiments with Stichopus was faintly alkaline to neutral red and neutral to tropäolin "000." Its C_{H}. was thus about 10^{-8}. Increasing the C_{H}. by the addition of acid (HCl)[18] led to the rapid cessation of pulsation movements.

Experiment 95.2. July 24. Sea water to which $\frac{N}{10}$ HCl had been added until just neutral to Congo red (i.e., C_{H}. = *ca.* $10^{-4.5}$) was tested with five fresh cloacal preparations. Pulsations of an irregular character were manifested for about 15 minutes in two of these tests. The anal sphincter did not pulsate, but remained about one-third closed.

In transferring the cloacal preparations to a desired solution, they were lifted out of sea water; they thereupon ceased beating (contracting as the result of mechanical stimulation)[19] and if returned to normal sea water about two minutes usually elapsed before pulsation began again. It was sought to utilize this fact and control the hydrogen-ion effect more closely (as was done by Dale and Thacker, '14, in analyzing the automaticity of different regions of the frog heart) by discovering the concentration which would just permit pulsation to begin and continue for about a minute. For the sphincter this concentration was attained by mixing equal volumes of sea water and sea water made just barely acid to Congo red: the mixture had therefore a C_{H}. of *ca.* 10^{-6}. This limit is very close to that found by Bethe ('09, p. 261) for the pulsations of medusae.

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[18] The effect of certain organic acids was also studied, but not with sufficient completeness to warrant discussion in this place.

[19] No pulsations were ever observed out of sea water.

Since it was not practicable to employ a perfusion method, experiments dealing with the action of the C_H. in solutions which permitted the continuance of pulsation for some time were not attempted, as it was found that such solutions were modified in the direction of neutrality by contact with the tissue for about half an hour.

It may be of interest to note that, although the function of cloacal pumping is in part respiratory, no increase in pulsation rate was induced by an increase in C_H.; cloacal rhythm in Stichopus thus resembles the (partly) respiratory movements of the arms of barnacles (Roaf, '12).

IX. SUMMARY

The rhythmic pulsation of the cloacal chamber and anal sphincter of Stichopus moebii is dependent upon the continuous generation of stimuli within the cloaca, and particularly at its anterior end. The mechanism whereby the radiating muscles of the cloaca, the circular muscles of the cloaca and anal sphincter, the anal dilators, and the muscles of the body wall are brought to act in orderly sequence in the pumping of water into the respiratory trees is likewise locally contained. The aboral ends of Stichopus moebii, Holothuria surinamensis, H. captiva, and Cucumaria punctata continue to pulsate for many hours after they have been amputated at the level of origin of the respiratory trees. In such amputated parts a complete pulsation movement begins with the opening of the anterior end of the cloaca; a wave of opening runs aborally along the cloaca; the anal sphincter then opens and afterward closes; a progressive constriction of the cloacal chamber begins at the closed anal sphincter and runs forward; at the termination of the pulsation the whole cloaca is closed.[20]

Normally the pulsation of the cloaca is interrupted by two means: (1) by complete constriction of the anal sphincter, in response to sensory stimulation and during locomotion, and (2) by holding the cloaca and anal sphincter open, during 'spouting.'

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[20] Doubtless the nervous arrangements for the production of this kind of sequence in movements is similar to that involved in the use of the lantern in the locomotion of Echinus (Gemmill, '12).

In the latter case the edge of the sphincter continues to pulsate. Pulsation continues during defecation. The isolated cloacal ends do not 'spout;' they contract in response to mechanical, chemical, and photic (shading) stimuli. The stimulus to spouting in the intact holothurian is probably derived from a condition of tension in the body wall.

The radiating muscles and connective-tissue strands of the cloaca are necessary for the performance of pulsation. They appear to act in connection with an integumentary nerve net.

The results of experiments upon the cloacal termination of Stichopus are in essential agreement with the data derived from many previous studies of pulsating structures, such as those of medusae, ctenophores, the arthropod heart, and the vertebrate heart and intestine. The rhythm has a temperature coefficient of the order of magnitude of that for chemical processes. The rate of pulsation is related to the size of the animal (in Stichopus, Holothuria surinamensis, H. captiva, and Cucumaria punctata) in such a way as to suggest that the larger animals, which pulsate more slowly, possess relatively less energy than do smaller ones of the same species. Pulsation of the amputated aboral end is readily depressed by urea, carbon dioxide, acids, and KCN; it is resistant to lack of dissolved oxygen. Either dilution or concentration of sea water curtails the duration of pulsation. Rhythmic movements do not continue for more than a few minutes in non-electrolyte solutions. The relation of pulsation to the salts of sea water is essentially like that in other well known pulsating systems; $NaCl + CalC_2 + KCl$ (in the proportions found in sea water) enables pulsations and irritability to continue longer than with NaCl or with $NaCl + CaCl_2$; but magnesium, and particularly $MgSO_4$ (at least in neutral salt mixtures), must be present to insure normal diastole. Calcium is intimately concerned in contraction. In the series

$$NaCl > LiCl > NH_4Cl > KCl \ (5/8\ M)$$

the preservation of pulsation and irritability is successively less. The pulsating mechanism is extremely susceptible to increase in the hydrogen-ion concentration. The addition of NH_4OH, or other alkalies, to normal sea water assists in the preservation of pulsation and irritability.

X. BIBLIOGRAPHY

Adrian, E. D. 1914 The temperature coefficient of the refractory period in nerve. Jour. Physiol., vol. 48, p. 453–464.

Alexandrowicz, J. S. 1913 Zur Kenntnis des sympathischen Nervensystems einiger Wirbellosen. Zeit. f. allg. Physiol., Bd. 14, p. 358–376., Taf. 12–13.

Alvarez, W. C. 1914 Functional variations in contractions of different parts of the small intestine. Amer. Jour. Physiol., vol. 35, p. 177–193.

Bethe, A. 1903 Allgemeine Anatomie und Physiologie des Nervensystems. viii + 487 p., 95 Fig., 2 Taf., Leipzig.

1908 Die Bedeutung der Elektrolyten für die rhythmischen Bewegungen der Medusen. Teil I. Die Wirkung der im Seewasser enthaltenen Salze auf die normale Meduse. Arch. ges. Physiol., Bd. 124, p. 541–577.

1909 Die Bedeutung der Elektolyten für die rhythmischen Bewegungen der Medusen. Teil II. Angriffspunkt der Salze, Einfluss der Anionen und Wirkung der OH- und H-ionen. Arch. ges. Physiol., Bd. 127, p. 219–273.

Bordas, L. 1899 Étude sur l'anatomie et les functions physiologiques des poumons aquatiques des Holothuries. Ann. Mus. Hist. Nat. Marseille, Zool., Tom. 5, Mém. 3, 15 p., 1 pl.

Cameron, A. T., and Brownlee, T. I. 1913 The effect of low temperatures on cold-blooded animals. Quart. Jour. Exp. Physiol., vol. 7, p. 115–130.

Cannon, W. B. 1911 The importance of tonus for the movements of the alimentary canal. Arch. Int. Med., vol. 8, p. 417–426.

Cannon, W. B., and Burket, I. R. 1913 The endurance of anemia by nerve cells in the myenteric plexus. Amer. Jour. Physiol., vol. 32, p. 347–357.

Child, C. M. 1913 Studies on the dynamics of morphogenesis, etc. . . . . V. The relation between resistance to depressing agents and rate of metabolism in Planaria dorotocephala and its value as a method of investigation. Jour. Exp. Zoöl., vol. 14, p. 153–206.

Clark, H. L. 1901 Bermudan Echinoderms. A report on observations and collections made in 1899. Proc. Bost. Soc. Nat. Hist., vol. 29, p. 339–345.

Crozier, W. J. 1914 The orientation of a holothurian by light. Amer. Jour. Physiol., vol. 36, p. 8–20.

1915 The sensory reactions of Holothuria surinamensis. Ludw. Zool. Jahrb., Abt. f. Physiol.

Dale, Dorothy, and Thacker, C. R. A. 1914 Hydrogen-ion concentrations limiting automaticity in different regions of the frog's heart. Jour. Physiol., vol. 47, p. 493–508.

Edwards, C. L. 1909 The development of Holothuria floridana Pourtalés with especial reference to the ambulacral appendages. Jour. Morph., vol. 20, p. 211–230.

Fosse, R. 1913 Présence de l'urée chez les Invertébrés et dans leurs produits d'excrétion. C. R. Acad. Sci., Paris, Tom. 157, p. 151–154.

Gemmill, J. F. 1912 The locomotor function of the lantern in Echinus, with observations on other allied lantern activities. Proc. Roy. Soc., London, Ser. B, vol. 85, p. 84–109.

Haanen, W. 1914 Anatomische und histologische Studien an Mesothuria intestinalis (Ascanius und Rathke). Zeit. wiss. Zool., Bd. 109, p. 185–255, Taf. 5, 6.

Henri, V. 1903 Étude des contractions rythmiques des vaisseaux et du poumon aqueux chez les holothuries. C. R. Soc. Biol., Paris, Tom. 55, p. 1314–1316.

Henri, V., et **Lalou**, S. 1903 Régulation osmotique des liquides internes chez les holothuries. C. R. Soc. Biol., Paris, Tom. 55, p. 1244–1245.

Hérouard, É. 1889 Recherches sur les holothuries des côtes de France. Arch. Zool. Exp. et Gen., sér. 2, Tom. 7, p. 535–704.

Höber, R. 1914 Physikalische Chemie der Zelle und der Gewebe. Leipzig und Berlin. 4te Aufl., xvii + 808 p.

Jordan, H. 1913 Eine neue Art von Muskeln. Proc. IX. Cong. Int. de Physiol. p.

1914 Über 'reflexarme Tiere.' IV. Die Holothurien, Mitteil. I. Die Holothurien als hohlorganartige Tiere und die Tonusfunktion ihrer Muskulatur. Zool. Jahrb., Abt. allg. Zool. u. Physiol., Bd. 34, p. 365–436.

Kafka, G. 1914 Einführung in die Tierpsychologie. Bd. I. Die Sinne der Wirbellosen. Leipzig. xii + 593 p.

Loeb, J. 1900 [a] Comparative physiology of the brain and comparative psychology. New York. x + 309 p.

1900 [b] On the different effect of ions upon myogenic and neurogenic rhythmical contractions and upon embryonic and muscular tissue. Amer. Jour. Physiol., vol. 3, p. 383–396.

1906 The dynamics of living matter. (Columbia Univ. Biol. Series, 8.) New York. xi + 233 p.

1910 The rôle of the concentration of hydroxyl-ions in the antagonistic action of K and Ca upon Na. Proc. Soc. Exp. Biol. Med., vol. 8, p. 10–11.

Ludwig, H. 1889–1892 Echinodermen. Bronn's Thierreich, Bd. 2, Abt. 3, Buch I. Die Seewalzen. Leipzig u. Heidelberg. vi + 460 p., 17 Taf.

Mark, K. L. 1913 Preliminary study of the salinity of sea water in the Bermudas. Proc. Amer. Acad. Arts and Sci., vol. 48, p. 669–678.

Mayer, A. G. 1906 Rhythmical pulsation in Scyphomedusae. Publ. Carnegie Instn. Wash., No. 47, 62 p.

1911 The converse relation between ciliary and neuro-muscular movements. Publ. Carnegie Instn. Wash., No. 132 (Papers from the Tortugas Lab., vol. 3), p. 3–25.

1914 [a] The effects of temperature upon tropical marine animals. Publ. Carnegie Instn. Wash., No. 183 (Papers from the Tortugas Lab., vol. 6), p. 1–24.

1914 [b] The relation between the degree of concentration of the electrolytes of sea water and the rate of nerve-conduction in Cassiopea. Publ. Carnegie Instn. Wash., No. 183 (Papers from the Tortugas Lab., vol. 6), p. 25–54.

Miller, F. R. 1910 On the rhythmical contractility of the anal musculature of the crayfish and lobster. Jour. Physiol., vol. 40, p. 431–444.

1912 Contributions to the physiology of the American lobster. The physiology of the intestine. Contrib. to Canadian Biol., 1906–1910, p. 277–280, pls. 30–35.

Moore, B., Edie, E. S., Whitley, E., and Dakin, W. J. 1912 The nutrition and metabolism of marine animals in relationship to (*a*) dissolved organic matter and (*b*) particulate organic matter in sea-water. Biochem. Jour., vol. 6, p. 255–296.

Moore, B., Edie, E. S., and Whitley, E. 1914 The nutrition and metabolism of marine animals: the rate of oxidation and output of carbon-dioxide in marine animals in relation to the available supply of food in sea-water. Rept. Lancashire Sea-Fish. Lab., No. 22, p. 297–320.

Pearse, A. S. 1908 Observations on the behavior of the holothurian Thyone briareus (Le S[u]eur). Biol. Bull., vol. 15, p. 259–288.

Piéron, H. 1909 La loi d'évanouissement des traces mnémoniques en fonction du temps chez la Limnée. C. R. Acad. Sci., Paris, Tom. 149, p. 513–516.

Polimanti, O. 1913 Sui rapporti fra peso del corpo e ritmo respiratorio in Octopus vulgaris Lam. Zeit. f. allg. Physiol., Bd. 15, p. 449–455.

Pütter, A. 1914 Temperaturkoeffizienten. Zeit. f. allg. Physiol., Bd. 16, p. 574–627.

Roaf, H. E. 1912 Contributions to the physiology of marine organisms. II. The influence of the carbon dioxide and oxygen tensions on rhythmical movements. Jour. Physiol., vol. 43, p. 449–454.

Robertson, T. B. 1910 Über die Verbindungen der Proteine mit anorganischen Substanzen und ihre Bedeutung für die Lebensvorgänge. Ergeb. Physiol., Jahrg. 10, p. 216–361.

Salant, W., and Hecht, S. 1915 The influence of oxalates, citrates and tartarates on the isolated heart. Amer. Jour. Physiol., vol. 36. p. 126–144.

Scott, J. W. 1914 Regeneration, variation and correlation in Thyone. Amer. Nat., vol. 48, p. 280–307.

Spaeth, R. A. 1913 The physiology of the chromatophores of fishes. Jour. Exp. Zoöl., vol. 15, p. 527–579.

Tashiro, S., and Adams, H. S. 1914 Comparison of the carbon-dioxide output of nerve fibers and ganglia in Limulus. Jour. Biol. Chem., vol. 18, p. 329–334.

Vernon, H. M. 1910 The mode of union of certain poisons with cardiac muscle. Jour. Physiol., vol. 41, p. 194–232.

Weiland, W. 1912 Zur Kenntnis der Entstehung der Darmbewegung. Arch. ges. Physiol., Bd. 147, p. 171–196.

Willey, A. 1899 Enteropneusta from the South Pacific, with notes on the West Indian species. Zoöl. Results of A. Willey, Part III, p. 223–334, Cambridge.

Winterstein, H. 1909 Über die Atmung der Holothurien. Arch. Fisiol., vol. 7, p. 33–40.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Moroulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908-
15. Moroulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 633–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. **Mark, K. L.** — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18): 669–678. April, 1913.
26. **Smallwood, W. M., and Clark, E. G.** — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23**(4): 625–636. Dec., 1912.
27. **Chester, W. M.** — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48**(20): 737–773, 4 pls. May, 1913.
28. **Kutchin, H. L.** — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49**(10): 569–626, 8 pls. Oct., 1913.
29. **Hilton, W. A.** — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1): 113–130. Dec., 1913.
30. **Crozier, W. J.** — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6): 491–492. Febr., 1914.
31. **Parker, G. H.** — The Locomotion of Chiton. 2 p. April, 1914.
32. **Parker, G. H.** — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16**(3): 443–445. April, 1914.
33. **Crozier, W. J.** — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **Crozier, W. J.** — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1): 8–20. Dec., 1914.
35. **Crozier, W. J.** — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577): 28–36. Jan., 1915.
36. **Arey, L. B.** — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1): 37–44. July, 1915.
37. **Crozier, W. J.** — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2): 196–202. Jan., 1915.
38. **Mavor, J. W.** — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9): 183–211, 6 pls. Dec., 1915.
39. **Crozier, W. J.** — On Cell Penetration by Acids. Science, N. S., **42** (1090): 735–736. Nov. 19, 1915.
40. **Crozier, W. J.** — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1): 1–8. Feb., 1916.
41. **Crozier, W. J.** — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3): 255–279. Mar., 1916.
42. **Hecht, S.** — The Water Currents produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3): 429–434. Apr., 1916.
43. **Crozier, W. J.** — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3): 297–356. Apr., 1916.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2): 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. Moroulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. Esterly, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

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29. **Hilton, W. A.** — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
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41. **Crozier, W. J.** — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
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43. **Crozier, W. J.** — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3) : 297–356. Apr., 1916.

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No. 44.

SOME INDICATORS FROM ANIMAL TISSUES.

By W. J. Crozier.

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SOME INDICATORS FROM ANIMAL TISSUES.

BY W. J. CROZIER.

Contributions from the Bermuda Biological Station for Research, No. 44.

(Received for publication, February 2, 1916.)

Indicators occurring in nature have for the most part been found in plants; in fact such materials have seldom been recorded from animal sources. An examination of a number of pigmented animals, however, shows that this rarity is by no means so great as has been supposed. The following notes refer to preparations obtained from some marine animals, which give clear-cut[1] reversible color changes as summarized in Table I. The hydrogen-ion concentrations observed were obtained by acetate, borate, and phosphate mixtures, at 20–25°, with the usual indicators for comparison (Michaelis, 1914). The tests were made by

TABLE I.

Color Changes of Some Indicators from Animal Tissues.

Source.	Acidity.*								
	$p_H = 5.0$	5.3	5.6	5.9	7.4	7.6	7.65	8.0	10.0
Ascidia atra......	Red.	(Increasingly red.) ←			Red.	Green (ppt.).			
Ptychodera sp......							Yellow.	Brown.	
Chromodoris zebra			Pink.	Blue.	Blue.	Blue.	Blue (green ppt.).		
(?) *Eupolymnia aurantiaca*......								Red.	Green.

* Observations were made at a number of intermediate points.

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[1] A number of other soluble pigments have been found in which an acid-alkali color change is undoubtedly present, but is not sufficiently clear-cut to be of use. For example, a sponge, *Tedania ignis*, gives brown extracts, which are made distinctly red by acids.

adding two drops of a concentrated solution of pigment to 10 cc. of the different mixtures.[2]

1. Ascidia atra.—This common black tunicate of Bermuda and the West Indies contains in its test a substance readily extracted by acetone, less easily by alcohol or glycerol, but not dissolved by ether or chloroform. This pigment is for the most part closely packed in the outer tenth of the thickness of the test, and occurs in the form of rather large granules carried by cells in the test canals. Its color is a deep purple, as may be seen when prepared sections of the test are studied microscopically. The pigment is insoluble in sea water, but observations indicate that it is excreted in granular form from the surface of the animal. According to Dahlgrün (1901), in many tunicates an excretory function is maintained by wandering mesenchymal cells the protoplasts of which bear pigment granules.

Solutions of the pigment are dark red, this color being intensified by acids and turned green by alkalies, which also precipitate the substance in greenish flocculent masses.

There is probably some relation between this dark purplish red substance and the pigment, described by Herdman (1913) and Holt (1914), which occurs in the test of the compound ascidian *Diazona violacea*,[3] although the *Ascidia* pigment does not appear to show any absorption bands.

2. Ptychodera sp.—This yellowish balanoglossid when strongly stimulated secretes a yellowish material which turns reddish brown at about $p_H = 8.0$. Extracts of the animal, containing this pigment, can be made with alcohol, distilled water, or aqueous solvents, but they are not useful as indicators because the brown color is permanently assumed on standing for some time. But within the tissues of the living animal the change resulting from the penetration of alkalies is well shown.

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[2] Useful indicators have also been prepared from several Bermuda plants. as the berries of the sage bush (*Lantana involucrata*), which yield on extraction with alcohol a bright red solution, changing to yellow (alkaline) at $p_H = 6.6$; the fruit of the cactus (*Opuntia*) also contains a red material changing to lemon yellow (alkaline) at $p_H = 9.0$; the juice from the ink berry of the sand beaches (*Randia aculeata*) is a splendid indicator, being dark red with acids and changing to a brilliant green (alkaline) at $p_H = 8.5$.

[3] Coloring matter precisely like that of *Diazona violacea* occurs in Bermuda ascidians which are closely related to this species.

3. Chromodoris zebra.—The indicator contained in this nudibranch has been described elsewhere (Crozier, 1914, 1915, 1916), but for comparison its color changes have been included in Table I.

4. Eupolymnia aurantiaca (?).—An orange red annelid, probably *Eupolymnia aurantiaca*, which is occasionally found among blocks of coral rock in which it burrows, gives with aqueous alcohol a bright red extract of the integument, which changes to orange on standing for some days. It shows a sharp transition from red to bright green at about $p_{\mathrm{H}} = 10.0$, though even at higher acidities a precipitate (which consists of the pigment substance) appears slowly; but the color change in the solution appears only above $p_{\mathrm{H}} = 10.0$. The red solutions transmit only a narrow portion of the red and of the yellow, while the green acid solutions transmit only the green-blue.

REFERENCES.

Crozier, W. J., 1914, Note on the Pigment of a Bermuda Nudibranch, *Chromodoris zebra* Heilprin, *J. Physiol.*, xlvii, 491.

Crozier, W. J., 1915, On Cell Penetration by Acids, *Science*, xlii, 735.

Crozier, W. J., 1916, Cell Penetration by Acids, *J. Biol. Chem.*, xxiv, 255.

Dahlgrün, W., 1901, Untersuchungen über den Bau der Excretionsorgane der Tunicaten, *Arch. mikr. Anat.*, lviii, 608.

Herdman, W. A., 1913, *Funiculina quadrangularis* (Pallas) and the Hebridean *Diazona violacea* Savigny (*Spolia Runiana*, I), *J. Linn. Soc. London, Zool.*, xxxii, 163.

Holt, A., 1914, The Colouring Matters in the Compound Ascidian *Diazona violacea* Savigny, *Proc. Roy. Soc., Series B*, lxxxviii, 227.

Michaelis, L., 1914, Die Wasserstoffionenkonzentration, Berlin, 210 pp.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole, L. J.** — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8): 315–328, pls. 20–22. July, 1904.
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4. **Coe, W. R.** — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–26. Nov., 1904.
5. **Bigelow, H. B.** — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. **Mark, E. L.** — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **Barbour, T.** — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. **Blackman, M. W.** — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. **Congdon, E. D.** — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. **Blackman, M. W.** — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. **Linton, E.** — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. **Parker, G. H.** — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts and Sci., **43**(16): 413–455. Apr., 1908.
13. **Cole, J. F.** — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. **Morgulis, S.** — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. **Morgulis, S.** — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
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38. **Mayor, J. W.** — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9): 183–211, 6 pls. Dec., 1915.
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24. **Smallwood**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

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35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577): 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1): 37–44. July, 1915.
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38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9): 183–211, 6 pls. Dec., 1915.
39. Crozier, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090): 735–736. Nov. 19, 1915.
40. Crozier, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1): 1–8. Feb., 1916.
41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3): 255–279. Mar., 1916.
42. Hecht, S. — The Water Currents produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3): 429–434. Apr., 1916.
43. Crozier, W. J. — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3): 297–356. Apr., 1916.
44. Crozier, W. J. — Some Indicators from Animal Tissues. Jour. Biol. Chem., **24** (4): 443–445. Apr., 1916.

59.14:51.7:(729.9)

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No. 45.

STUDIES ON A PHOSPHORESCENT BERMUDIAN ANNELID, ODONTOSYLLIS ENOPLA VERRILL.

By T. W. Galloway and Paul S. Welch.

With Five Plates.

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CAMBRIDGE, MASS., U. S. A.
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STUDIES ON A PHOSPHORESCENT BERMUDAN ANNELID, ODONTOSYLLIS ENOPLA VERRILL.[1]

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I. INTRODUCTION AND NATURAL HISTORY.

During the summer of 1904 the senior author, while working at the Bermuda Biological Station, had the opportunity to observe with some care two appearances of a remarkably interesting phosphorescent annelid.[2] The laboratory was at that time located at Hotel Frascati on the Flatts Inlet to Harrington Sound. The tide runs freely into and out of the Sound by way of the Inlet. The worms appear periodically in the waters of this inlet in considerable numbers and with striking regularity. It was reported to me that they also occur in the waters of St. George's Harbor. In all likelihood they are to be found at numerous points about the Islands.

Two appearances were observed by me at Frascati, and a third was reported to me. The first occurred July 3–7, with a maximum on the 4th; another July 29–31, with a maximum on the 30th. The latest appearance was reported to me as occurring on August 23; but details are lacking as to its duration. There is thus an interval of about 26 days between these maxima.

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[1] Note.— Owing to a misunderstanding, this paper, originally printed in the Transactions of the American Microscopical Society, Vol. 30, No. 1, did not appear as a Contribution from the Bermuda Station. It is now reprinted, with the authors' approval, and assigned a number in those Contributions.— E. L. MARK.

[2] The indebtedness of the writers is hereby acknowledged for courtesies extended by the Bermuda Biological Station (supported by the Bermuda Natural History Society and Harvard University) and by Professor Edward L. Mark, its Director. The identification of the worm was made by Dr. J. Percy Moore of the Academy of Natural Sciences of Philadelphia.

The full meaning of this interval is not clear. The lunar month is, however, at once suggested. This would in all probability come to be established through the tides, either alone and directly, or in connection with light variations produced by tidal variation. These agencies might possibly become operative in two ways: (1) in connection with the formation of the sexual cells, and (2) in their release. How the effects are wrought in this case, we are not in a position to say. The matter should receive more careful local study.

According to the tide tables, the tide was at its lowest for the day in the Bermudas on July 4 about 6:30 P. M., the moon being in its last quarter on July 5th. On July 27th was a spring tide; and the second recorded appearance began July 29th. On July 31 there was a near-spring tide at its lowest daily phase at 7:30 P. M. While these two appearances do not show a strict parallelism with the lunar phases, they do involve a coincidence of low tides and approaching dark.

If my informant was correct in his dates, the third appearance would occur at high tide. It is possible that the first appearance of the season, involving a release of gametes, is stimulated by low tide or by the coincidence of low tide and approaching dark; that the time necessary for renewal of reproductive bodies has been established at approximately the lunar month by a series of circumstances, internal and external; and that the release of the sexual products occurs on the first approach of dark after their maturity irrespective of the height of the tide at the time.

Parallel instances of periodicity in the formation and release of sexual bodies are numerous, in which both tide and light appear to play a part.

In addition to the monthly periodicity, there is a daily periodicity. On both occasions on which they were carefully observed they appeared each day, within fifteen minutes of the same time, just as dusk was becoming pronounced.

The display lasted from twenty to thirty minutes. Only a few appeared at first, each evening. The numbers gradually increased to a maximum, when scores might be seen at once. The display waned somewhat more rapidly than it waxed. An occasional belated specimen sometimes appeared some minutes afterward.

In a similar way, not so many individuals were seen on the first evening of each period. On the second or third night they reached a maximum, and again dwindled in numbers on following days.

Of the three appearances, that of early July was the most numerous, and that of August the least so. This suggests an annual, as well as a daily and monthly maximum; but this needs further observation.

The males and females differ considerably in size — the females often being twice as long as the males. The larger female specimens attain a length of 35 mm. Both sexes are distinctly phosphorescent: — the female with strong and more continuous glow, and the male with sharper, intermittent flashes.

In mating, the female, which is clearly swimming at the surface of the water before it begins to be phosphorescent, shows first as a dim glow. Quite suddenly she becomes acutely phosphorescent, particularly in the posterior three-fourths of the body, although all the segments seem to be luminous in some degree. At this phase she swims rapidly through the water in small, luminous circles two or more inches in diameter. Around this smaller vivid circle is a halo of phosphorescence, growing dimmer peripherally. This halo of phosphorescence is possibly caused by the escaping eggs, together with whatever body fluids accompany them. At any rate the phosphorescent effect closely accompanies ovulation, and the eggs continue mildly phosphorescent for a while. The fact that the luminosity is known at no other time is further suggestive that it is produced by the material which escapes from the body cavity. If the phosphorescent glands are external, as the histology of the epidermis at least suggests, the discharge of the glands is closely correlated with ovulation.[3]

If the male does not appear, this illumination ceases after 10 to 20 seconds. In the absence of the male the process may apparently be repeated as often as four or five times by one female, at intervals of 10 to 30 seconds. The later intervals are longer than the earlier. Usually, however, the males are sufficiently abundant to make this repetition unnecessary; and the unmated females are rare, if they are out in the open water. One can sometimes locate the drifting female between displays by the persistence of the luminosity of the eggs; but the male is unable to find her in this way.

The male appears first as a delicate glint of light, possibly as much as 10 or 15 feet from the luminous female. They do not swim at the surface, as do the females, but come obliquely up from the deeper water. They dart directly for the center of the luminous circle and they locate the female with remarkable precision, when she is in the acute stage of phosphorescence. If, however, she ceases to be actively phosphorescent before he covers the distance, he is uncertain and apparently ceases swimming, as he certainly ceases being luminous, until she becomes phosphorescent again. When her position becomes defined he quickly approaches her, and they rotate together in somewhat wider circles, scattering eggs and sperm in the water. The period is somewhat longer on the average than when the female is rotating alone; but it, too, is of short duration.

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[3] Postscript.— For a discussion of the source of luminosity in these worms, see Dahlgren's recent paper: "The Production of Light by Animals." Jour. Franklin Inst., 181, No. 5, pp. 659–696. May, 1916.

So far as could be observed, the phosphorescent display is not repeated by either individual after mating. Very shortly the worms cease to be luminous and are lost. Often they give the appearance of sinking out of sight; however, this appearance is negatived by the fact that I have caught both sexes at once by timing the current and dipping down stream, as much as six or eight feet from the point of latest visible phosphorescence. Sometimes as many as two or three males seem to take part in one mating.

The females caught and examined immediately on becoming luminous are full of eggs. Those caught after three or four displays, or after copulation, are largely empty of eggs; yet the different segments of one worm will differ widely in this particular. Eggs are often caught among the setæ and at any other points where they can be held.

Specimens in confinement after copulation may be aroused into mild phosphorescence for at least an hour.

The group of mating adaptations in this Syllid is peculiarly large and complex; and the elements entering into the precision with which the eggs and sperm are brought together are quite worth noting. In a number of counts made of eggs captured in connection with the copulating worms, I found a range of 45–80 per cent of fertilized eggs in five batches taken at random. Considering the external fertilization of the eggs this must be considered very high. It is quite probable that this is a higher result than would be attained if the eggs and worms had been left in the sea.

The following correlated adaptations are noteworthy:

1. The concentration of the ripening and production of the ova and sperm into a few days of each month, in the worms of a given locality; and the coincidence of the periodicity of the male and female.

2. The further concentration of these processes into 30 minutes of the twenty-four hours, and at the coming of darkness.

3. The coincidence of the luminosity with the emission of eggs and, so far as we know, its confinement to this period.

4. The repeated periods of luminosity of the female — serving as an adequate lure for the male, even though his distance may be considerable.

5. The sex-dimorphism in the character of the flashes in male and female, which may serve as recognition marks.

6. The fact that the females swim at the surface of the water at this time, while the males are beneath the surface until the former become luminous, enables the males to locate the females with greater precision. This position of the female may also make the oxidation more complete, and thus secure the increased luminosity.

7. The eyes of the male are perceptibly larger that those of the female, in spite of the fact that the females are distinctly larger than the males.

II. CLASSIFICATION.

The Syllidae, of which *Odontosyllis* is a genus, are an interesting and widely distributed family of annelids. While nothing is known of the habits and manner of life of this species of *Odontosyllis* except what is seen in this mating period, there are numerous striking features of the family recorded. They are mostly free-swimming forms; but it is believed that they dwell largely amidst the fixed vegetable and animal growths between the tide-marks, or at shallow depths, and seek their food there. Some species are known to be commensal with sponges. Certain species of *Autolytus* are reported to be parasitic on nemerteans, and on other species of Polycheta. In such cases the proboscis is said to be incapable of being retracted.

To the general student the most remarkable facts about the family relate to the methods of reproduction. As in some of the naidiform Oligocheta, non-sexual reproduction by "budding" is a common occurrence. This budding may be of the nature of transverse fission, as in the naidiform worms; or it may be lateral, sometimes even with rosettes of buds rising from the side of the body. In one species of *Autolytus* the budding may continue from these primary buds in such a way as to produce a complex, much branched stock very similar to plant growth. As will be recognized, this is an uncommon occurrence among animals as highly differentiated as the Syllids. In *Autolytus* and some other genera, possibly in many of the genera, a non-sexual nurse-stock gives off numerous sexual buds or zooids, which ultimately escape and mate as free-swimming worms. The embryos develop into the nurse-stock and thus a somewhat complicated alternation of generations comes about.

Malaquin (1893) diagnoses the family Syllidae as follows:

> "Cephalic segments provided with 5 appendages: Namely, two palpi; two lateral and one median antennæ; and two pairs of eyes. The peristomial (post-cephalic) segment usually has two pairs of tentacle-like cirri,— sometimes only one pair. The succeeding segments have feet consisting only of the setigerous lobe of the ventral division, together with a dorsal and a ventral cirrus. The dorsal division of the foot often develops at the time of sexual maturity. The proboscis is protrusible, and consists of two regions: (1) the anterior (pharynx) chitinous and with one or more teeth; (2) the muscular gizzard, which is a secondary development of the pharynx of the larva. Reproduction is distinguished by the appearance of secondary sexual characteristics (such as enlargement of the eyes, elongation of the antennae, development of swimming bristles and of genital glands, and often of phosphorescent organs). The ordinary individual may thus itself become sexual by these changes (epigamy); or it may give rise to new and special buds which separate and assume the sexual characteristics (schizogamy)."

The Syllidae are divided into the following sub-families:

Syllidae.	Palps present	Fused	Thruout	Exogonea.
			At base only	Eusyllidae.
		Separate		Syllidae.
	Palps wanting			Autolytea.

Malaquin defines the Eusyllidea, to which *Odontosyllis* belongs, thus:

> "Syllidae with ventral cirrus (McIntosh says that these may be absent; they are wholly wanting in both sexes of the worm under consideration); palpi fused at the base only. Tentacular cirri filiform and cylindrical, with surface constrictions. Reproduction epigamous (direct)."

Odontosyllis was established as a genus by Claparède (1863) and is described as follows:

> "Palpi short or moderately elongated; more or less separate or fused at the base. Tentacles (3) and the dorsal cirri filamentous, short — becoming longer in sexually mature zooids. Nuchal organ has a central pit. Tentacular cirri in two pairs. Ventral cirrus present [not in the sexual zooids of *O. enopla*]. Proboscis with a series of horny papillæ, the points curved backward. Ventricle (stomach) short and devoid of T-shaped cæca. Bristles with the terminal piece simple or bifid."

Verrill (1900) has described *Odontosyllis enopla* as follows:

"A large species with a dark brown, wide, short esophagus, armed with a ventral row of six stout, recurved, hook-like teeth anteriorly, besides the median dorsal tooth.

Head large, broader than long, broadly rounded in front and on the sides; posteriorly with two rounded lobes, separated by small median emargination. Eyes black, unequal, the anterior ones much larger, reniform; those of each side are so close together that they seem to be almost in contact.

Palpi shorter than the head, rather wide, thin, often wrinkled or folded in contraction, and commonly curved downward.

Tentacle tapered, rather slender, not annulated, its length about 1½ times that of the head. Antennae similar, about ½ as long. Tentacular cirri similar to the tentacle, the upper one rather larger and longer; the lower ones shorter; first dorsal cirrus decidedly longer and larger than the upper tentacular cirrus. Succeeding ones mostly shorter, unequal, alternately shorter and longer, tapered distally; the longer ones are equal to the breadth of the body, the shorter ones about ½ as long; those on setigerous segments 3, 4, 6, 9 are longer than the others.

The setæ are all similar, numerous, slender, short, projecting but little beyond the parapodia, with short, rather wide blades, ratio as 1:2½–3; their tips are strongly incurved and acute, with a small denticle a little distant from the end [Fig. 16, V]. Two spiniform yellow acicula usually occur in each fascicle.

The esophagus is short and occupies about 4 segments; its margin is incurved and strongly emarginate dorsally. It bears a group of 6 [see Figs 18, 19, 23] nearly equal, parallel, recurved hooks or teeth, which are large and strong. The conical dorsal tooth is near the margin.

The stomach is large and occupies 8 segments; it is wide, elliptical, and about twice as long as the esophagus. Its surface is covered with angular or alveolar markings, often hexagonal, so as to have a honeycomb-like appearance, but not arranged in definite rows.

Color, in formalin, is nearly white, except when containing eggs.

Length, 25 mm.; diameter, about 1.5 mm.

One of the largest specimens has all the segments back of the gastric region filled with eggs."

III. GENERAL AND EXTERNAL MORPHOLOGY.

As in the other nereidiform worms, the body is elongated and very mobile. The length varies in the observed specimens from 19 to 35 mm. The males are distinctly smaller than the females. Just back of the head the body is almost cylindrical. The dorso-ventral diameter grows continuously shorter from before backward, while the transverse diameter lengthens for about 40 segments, after which it, too, gradually diminishes.

The segments vary in number from about 110 to 130, in the twelve or fifteen specimens examined. They may be grouped in the following regions: (1) a head with 3 to 6 specialized segments including the first setigerous segment; (2) an anterior body region of 23 or 24 segments, in which there is a gradual increase in the right-left diameter, and upon which the dorsal portion of the parapodium (notopodium) bears no setae, although the ventral process (neuropodium) does; (3) a mid-region, comprising some 30 to 32 segments, and similar to the last segments of the preceding region but for the fact that each notopodium bears a cluster of long, swimming setae in addition to the neuropodial setae; (4) a posterior region, similar to the second region in that the notopodial setae are lacking, which includes all the rest of the worm, except — (5) the specialized anal segment, which bears beside the anus, a pair of elongated cirri. The variation in the number of segments in the worms is due to differences in the fourth region enumerated above. The length of the other regions is subject to very little variation.

The Head.

As is usual in the polychetes the head is well differentiated, and the problem of its segmentation is not an easy one. There is the usual pre-oral portion known as the prostomium; the mouth itself; and a region surrounding the mouth and just back of it, the peristomium. See Figs. 2, 5, 12, 13. This specialized head includes the first setigerous segment and everything in front of it. If the prostomium, as is held by Malaquin, consists of one segment only, the head of *Odontosyllis* contains four segments. If, as Pruvot thinks, the prostomial lobe represents a modification of three segments, there are six segments in the head.

If we regard the prostomium and its outgrowths as fully homologous with the structures in other regions of the body it would seem that Pruvot's contention is the sounder of the two. For the dorsal prostomial lobe bears three types of paired sensory structures: foremost of all, two ciliated palps; back of these and separated from them by a groove, the pair of lobes bearing the eyes, of which there are two on each side of the head; and, lying between these lobes, the three tentacles, one medium and two lateral (Fig. 12). It is not easy to see how this region can be regarded as segmented at all and consider it of less than three segments; but in the light of the embryonic development of this and similar worms it is questionable whether it is sound to try to homologize the divisions of this prostomial organ or even the whole organ with the segments of the body. In the larva of *Odontosyllis*, as in other Syllids, segmentation is distinctly a secondary state, superimposed on the posterior part only of the trochosphere. The anterior enlarged portion seems not to share in this embryonic segmentation. In our opinion the prostomium rather represents a modification of this unsegmented anterior outgrowth of the trochosphere. It is not even morphologically a whole segment; but is rather an outgrowth and specialization of a portion of the first embryonic segment.

The peristome, on the other hand, is clearly of three segments, each bearing a pair of lateral cirri becoming progressively more dorsal. Only the third of these segments (posterior) appears, however, as a complete ring of the body. The dorsal part of this segment may protrude in the form of a flap covering the base of the lobes bearing the eyes (Figs. 1, 13, *F*). In life this is quite distensible. A ventral projection of this same segment forms the lower lip (Fig. 5). The first and second segments show only as partial rings extending from the side of the mouth to the over-arching prostomium.

The Eyes.

The four eyes are arranged in pairs, two eyes on either side of the median line (Figs. 1, 12, 13, *Ey*). They are mounted on protruding lobes, which are capable of a certain amount of motion. The anterior eyes are somewhat larger than the posterior ones, though not markedly so. The eyes of the males are distinctly larger than those of the females. In measurements of the eyes of two males and three females, after making allowance for differences in planes of sectioning, there is a difference of 10–30 per cent in favor of the males. The opening into the pigment cup (pupil) of the anterior eyes in the normal position is directed forward and outward; that of the posterior ones, dorsally (Fig. 1, *E*).

The cuticle which covers the front of the eye is a continuation of that which covers the exterior of the body. The lens is a spherical body lying in the cavity formed by the pigment cup (Fig. 7, *Le*). It appears to be connected with the cuticle through the opening in the pigment cup, by a slender stalk or pedicel. After fixation the lens shows as a somewhat fibrous substance surrounding at various places spicuous nuclei near the bases. The basement membrane is very definite in all regions, staining readily with haematoxylin. In certain quite definite regions these cells vary from the common shape. They become much more elongated near the base of the parapodium on both dorsal and ventral sides. The greatest thickening takes place, however, in the region just in front of the dorsal flap that extends forward from the last peristomial segment, as far as the base of the brain (Fig. 6, *E′*). At this point the epidermal cells are tremendously elongated — the length in the longest being something like fifteen times the short dimension, and 6 or 8 times as long as is usually the case. This patch of cells has all the appearance of a sensory epithelium. There are several such patches, less striking, about the various flaps and folds of the head.

The regular glands are rather numerous, and are found in all regions of the skin. They seem to be a little more numerous on the sides of the segments above the bases of the parapodia, and on the ventral surface to the right and left of the median groove. These cells have a characteristic shape resembling a truncated cone, the larger diameter being distal. The cell-wall, nucleus, and cytoplasm are quite distinct, and the latter has a very characteristic reticulated structure which is shown in Fig. 15.

The third type of epidermal cells — the "twisted" gland cells — occur in most parts of the epidermis, and are rather numerous. They occur in the ordinary regions of the body singly or in small groups separated by one or more epithelial cells; but they are often in clusters of 4 or 5, or more, on the tentacles and cirri. In general they are more numerous on the exposed parts of the body, and much less so in the depressions, though they are abundant at the lips of such grooves. They are somewhat flask-shaped, usually with irregular surface depressions that give the appearance of a spiral twist (Fig. 22). They vary considerably in size. Each cell has a distinct neck, and opens to the exterior through a pore in the cuticle. The reticulum is conspicuous throughout the cell. Usually these cells stain densely, especially at the wall. The internal reticulations are very much less stained. Often the glands show only a slight, ghostly staining, suggesting a difference of physiological state. The distribution, and the apparently empty condition of many of the glands suggest that they may be the phosphorescent organs; though of this the authors have no final proof. In the dorsal region of a male specimen these glands show a somewhat different structure, as appears in Fig. 22, *Sg*. Here the structure is of a much divided tubular sort. Their distribution and general relations, however, mark them as identical with the twisted cells.

The muscles of the body-wall present no significant departure from the condition described for other nereidiform worms. The outer circular layers give rise to acicular muscle fibers and to fan-shaped oblique fibres concerned in the motions of the parapodia. The longitudinal muscles are massed in four heavy bands — two dorsal and two ventral. They, too, give off tracts of fibres to the parapodia. The general relations of the muscles are very well shown in Figs. 20, 21. The muscles are unstriate.

The Alimentary Canal.

The alimentary canal is a nearly straight tube running the length of the body from the mouth to the anus. It is in no wise degenerate, as in some of the Syllids; but is functional throughout its course. Five regions may be distinguished, the last four of which are sharply differentiated from each other: (1) buccal cavity; (2) pharynx; (3) esophagus; (4) gizzard; (5) intestine.

A number of features, both embryonic and histological, seem to suggest the point of union of gizzard and intestine as the beginning of the mesenteron. There is a cuticular lining with very pronounced developments as far as the anterior end of the gizzard, and in less degree throughout its extent. It will be seen that the chief differentiations of the tract are in the stomodaeum, the mesenteron being quite uniform in size and structure.

The Mouth and Pharynx.

The first two regions, the buccal cavity and the pharynx, constitute an introvert. When withdrawn the buccal cavity occupies the peristomium, and the pharynx extends from the first setigerous segment to the seventh. When fully everted the buccal region is turned wrong side out, and the terminal opening is directly into the muscular pharynx, which is pulled forward its full length so that its posterior end is in the first or second setigerous segment. Compare Figs. 2 and 3.

The oral opening, when the introvert is withdrawn, presents a lobed margin of which the first setigerous segment furnishes the posterior ventral portion — a kind of lower lip (Fig. 5). The first and second peristomial segments form the remainder of the boundary to the mouth. The ventral floor of the buccal cavity is glandular (Figs. 2 and 14, *g*), while the sides are merely transitional from the outside to the pharynx. There is also a deep glandular fold from the dorsal wall of the cavity at the anterior margin of the pharynx (Figs. 1, 2, *O*).

The anterior part of the pharynx is very thick and muscular, but the posterior one-third, or thereabouts, is thin and pliable. It is this latter region that allows the adjustment of the pharynx to the body space when it is withdrawn into the body. This thin zone is thrown into a fold directed backward into the coelom, around the anterior end of the esophagus (Figs. 2, 3, *Ph'*). The anterior end of the esophagus thus comes to lie within the lumen of the posterior part of the pharynx. The wall of the pharynx is composed of (1) the internal cuticle, (2) the epithelial lining, which secretes the cuticle, (3) a thick muscular coat, and (4) the peritoneum.

The cuticle is continuous with that of the skin and similar to it in structure, but presents modifications in certain regions. It is thickened into minute conical denticles over a large portion of the pharyngeal surface (Figs. 2, 14, *dt*). In the dorsal part the denticles begin with the mouth and extend about one-third of the length of the pharynx. In the ventral floor they begin at the posterior edge of the glandular region (Fig. 14) and cover perhaps two-thirds of the floor back of that point. The glands and the denticles do not occur together.

The epithelial layer of the pharynx is thrown into a series of longitudinal folds, somewhat irregular at first, but becoming more definite posteriorly. One of these in the dorsal part of the pharynx has almost the definiteness of form of the typhlosole of the intestine of the earthworm (Fig. 18). The epidermal cells of the posterior portion of the thick muscular region of the pharynx are conspicuously glandular. In the thin region, that forms the flexure in introversion, the cells revert more nearly to the ordinary type of epithelium.

The muscular layer is the most conspicuous one in the wall of the pharynx. It consists of a complex of circular, longitudinal, and radial or oblique fibres. In a longitudinal section of the organ (Fig. 2) the circular fibres show themselves to be arranged on both sides of sheets which radiate from the lumen outward not at right angles to the lumen, but in positions depending on the degree of inversion. The circular fibres appear to dominate in this organ, but it is much less easy to distinguish the various layers here than in any other part of the body. Indeed it is easy to see that the layers are not as independent as elsewhere, and intermix to a greater degree.

The Esophagus.

The esophagus when withdrawn occupies segments 8 to 11. At its anterior margin occurs a ring of cuticular teeth which give the name to the genus. In the ventral floor there is a row of six of these, closely appressed, uniform in size, conical in form, and curved slightly backward. On the lateral walls near the dorsal part, and arching over so as to engage the ventral teeth, is a somewhat larger recurved tooth on either side of the lumen. In introversion the dorsal ridge of the pharynx is pressed in between these lateral teeth (see Fig. 18, *Df*). It is very difficult to get satisfactory sections in the region of the teeth.

From within outward the layers in the wall of the esophagus are: The very much thickened cuticle, comprising about one-third the thickness of the whole wall; the columnar epithelium; the layer of circular muscles, about the thickness of the epithelium; the longitudinal muscles, two-fifths the total thickness of the wall; and the thin peritoneum.

The cuticular layer is thicker in the esophagus than at any other part of the body. It stains densely except at the very outer margin and shows a clear striate structure, the striations running perpendicular to the surface. While the teeth are to be looked upon as special thickenings of the cuticle, there is a sharp demarcation between this layer and them (Fig. 23).

The arrangement and character of the muscles in the region of the esophagus show that they are not so much concerned with the mere action of the esophagus itself as with the larger problem of introversion and eversion, and of the manipulation of the teeth at the anterior end of the esophagus. It is not the purpose of this paper to trace out the course and value of the various muscular tracts; but an examination of the various figures will suggest the rôle of some of the muscles massed about the anterior end of the esophagus. They bring the latter organ into proper relation to the processes being initiated by the action of the pharynx, as well as furnish a point of attachment for some of the elements engaged in retraction and protrusion. The thickened cuticle of the esophagus is undoubtedly for this purpose, and for the added purpose of keeping the esophagus open and functional during the various vigorous changes of form and position of the introvert.

The Gizzard.

The gizzard, or stomach, is a highly developed structure immediately following the esophagus and sharply demarcated from it. It extends normally from about the 12th setigerous segment to the 21st. It practically fills the body cavity in these segments. It is of uniform size except where it tapers off rather abruptly to join the esophagus and the intestine. It is elliptical in cross-section; and the lumen is in the form of a long narrow slit, the sides of which are nearly parallel. This slit-like lumen divides the organ into symmetrical halves, whose walls are very thick except in the mid-dorsal and mid-ventral regions. Here it is not more than 1–5 the usual thickness. Apparently the morphological position of the slit is dorso-ventral; but the whole organ may be rotated until it has a right left position. From a surface view, or in excentric longitudinal (tangential) section, the whole wall of the organ is seen to be crossed by a series of fine parallel lines running transverse to the organ. The band-like spaces between these lines are divided into numerous angular areas of nearly uniform shape and size. The whole presents a remarkably regular and interesting pattern (Fig. 25). Haswell (1886) has shown in his study of various species of Syllids that the wall of the gizzard is a highly complex muscular organ, and is not, as has long been supposed, a glandular one at all. These studies confirm his conclusion in all essential particulars.

In detail, the wall of the gizzard, from within outward, shows the following regions: (1) the epithelium with its very thin secretion of cuticula; (2) a very much reduced muscular layer (Fig. 24, *mi.*), which contains internally one thickness of circular fibres and just without this a similar single thickness of longitudinal fibres; (3) a thick zone made up of muscular elements, which show chiefly as radiating columns in a transverse section or in longitudinal sections radial to the organ (Figs. 21; 2, V; 24, *Col*); (4) a thin layer of elements (Fig. 24, *mo*) which certainly contains circular muscular fibres, and according to Haswell contains longitudinal fibres also; (5) the thin peritoneal layer.

None of these layers call for special comment with the exception of the thick muscular layer which furnishes the main body of the wall of the gizzard.

A view when the gizzard is cut longitudinally, in such a way as to display the radial elements uncut, would be illustrated by Fig. 2, V, at the left-hand side of the figure. Such a view shows three chief topographic features: (1) a series of fibrous columns passing from the thin inner muscular sheet to the outer; (2) lying between these, open spaces somewhat shorter than the columns and tapering toward both ends; and (3) in the outer portion of these open spaces, tapering objects, thicker at the outer ends, which extend from the outer wall about one-half the way to the lumen.

Study of these objects in other sections (see Figs. 2; 24) shows the radiating columns to be muscle fibres extending the whole thickness of the wall of the gizzard. They appear as columns in the transverse section of the gizzard also (Fig. 21, *3*), but do not have such well-developed spaces between them. The tapering objects extending inward between the columns present a granular appearance and are in fact annular muscle fibres cut cross-wise (Fig. 28, *Cb*). They form the transverse lines that show in a tangential section, such as is seen in Fig. 25, *Cb*. They are in reality thin, wedge-like bands of muscle fibres that encircle the gizzard and separate the radial columns into successive circular zones, also shown in Fig. 26, *Cb*. Only the outer halves of the columns are thus divided, since the circular bands extend only part way to the lumen. In the cross-section of the gizzard these circular bands present their fibres in longitudinal view. They show dimly (Fig. 21, *2*) in such sections outside the wavy line which appears midway the columns (Figs. 21, 24). These circular fibres are of the usual unstriate type and coalesce with the circular sheet of fibres of the inner muscular layer at the dorsal and ventral edges of the lumen of the gizzard (Fig. 21, *Ra*), and only there. The wavy line seen in Fig. 24 about midway on the columns seems to be the mark of the union of the inner thin edge of the circular band of fibres with the adjacent radial columns.

The histology of the radial columns has been worked out in much detail by Haswell for several Syllids. The differences between the conditions in *O. enopla* and those described by him are of minor moment. The individual fibrils run the length of the columns and are undoubtedly made up of alternate light and dark bands, such as are seen in typical cross-striate muscles. Haswell remarks that the striations found in these fibres are more strongly marked than in any crustacean or insect he had examined.

Considered as a whole, each one of these radiating columns of cross-striate muscle fibrils is made up longitudinally of two symmetrical halves hollowed out on their inner faces in such a way as almost to surround a cavity. This cavity is small and closely surrounded near the inner end of the column, as seen in cross-section (Fig. 27, *f*); but in cross-sections further from the lumen, the space between the two halves becomes larger, and is less completely surrounded by the muscle fibres. In the outer half of its course the halves of a given column fall on different sides of one of the circular bands of muscles referred to above. See Fig. 26, which represents a cross section of a group of the columns about two-thirds of the way toward the outer surface, and shows the circular muscles separating the triangular halves of the columns and thus throwing them into a band which is back to back with the nearby halves of the next row of columns. Figure 25 shows the relation of the parts just as close to the outer end of the columns as a section can be made. In this figure the outer ends of the half-columns included between two of the bands of circular fibres are shown almost to run together into a continuous zig-zag sheet. At this level the space between the circular band of unstriate fibres (Fig. 25, *Cb*) and the adjacent zone of the radial columns (Fig. 25, *col*) is occupied by a mass of granular nucleated protoplasm (Fig. 25, *n*). This protoplasm extends only a short distance down the columns, as appears in Fig. 28, *n*, which shows a section perpendicular to that in Fig. 25.

In brief summary, then, the gizzard is a complex muscular organ with an outer and inner sheet of unstriate fibres; a parallel series of thin bands of annular unstriate muscles, the outermost fibres being in close relation to the external muscular layer, which extends inward as a band from the outer muscular sheet only about one-half the thickness of the whole wall, except that it reaches entirely to the inner muscular sheet at the angles of the lumen where the wall is thinnest and there gives off fibres which unite with it; and a series of radial columns of striate muscle fibres, each column of which is made of two trough-like halves whose concave sides are apposed in such a way as to make a conical cavity with its largest diameter at the outer end.

From the point of view of histogenesis the most interesting things about the gizzard are this mixture of striate and nonstriate fibres in the single organ, and the evidence that the central cavity of the radial columns presents at the outer end a remnant of the granular protoplasm from which the muscular fibrils were differentiated. Each of these hollow columns is a simple muscular organ, in which the contractile elements are the product of the single multinucleate mass of protoplasm occupying its core and it is differentiated progressively toward the periphery, as is suggested by the last position of the nucleated protoplasm (Fig. 28, *n.*). The embryonic character of the mature fibres, and the simplicity of the relation of the fibres in the organ mark the organism as one that would well repay study upon the ontogenetic differentiation of the muscles of the gizzard.

The Intestine.

The intestine is a straight tube passing with nearly uniform character, and with gradually diminishing size, to the anal opening. It has the usual constrictions between the segments, with sacculations almost amounting to diverticula within the segments. The epithelial lining has the usual variety of columnar cells. Those in the posterior part of the tract are heavily ciliated. Both circular and longitudinal fibres may be found, but they do not form a definite or continuous sheet of tissue. Fig. 20 illustrates a cross section of the body, in the intestinal region, which includes a dissepiment. The intestine is sharply constricted; the layer of longitudinal muscle fibres appears clearly just outside the entoderm; and a thick zone of circular fibres outside this shows a clear anastomosis with the circular fibres of the body wall in both dorsal and ventral regions. The distribution of oblique muscle fibres in the dissepiment is also shown — the origin being in the ventral region on either side the nerve cord, whence the fibers pass out fan-wise to the muscular layers of the dorso-lateral walls. At least some of these fibres run into the circular muscular sheet in being inserted.

The only noteworthy differentiations in the length of the intestine are the shallow proctodeum, and the valvular enlargement at its junction with the gizzard (Fig. 4, *V*). At the beginning of the intestine there is a special annular or sphincter group of muscle fibres (Fig. 4, *S*).

The Circulatory System.

The blood vessels agree with those described as characterizing the Syllids generally, and call for little special discussion. The dorsal vessel is small and thick-walled as compared with the ventral.

The dorsal vessel is imbedded in the wall of the intestine in an interesting way through a part of its course — the peritoneal membrane inclosing both in one sheath (Figs. 17; 29). Segmental blood vessels arise from both the dorsal and ventral longitudinal vessels and run outward on the anterior face of each dissepiment.

The Excretory System.

The nephridia are quite large organs in this worm in comparison with the size of the body. This is due in the female to the liberal glandular portion. The nephridia apparently occur in all the setigerous segments of the body beginning with the very first, which is also the last segment of the peristomium.

In the male the tubular ciliated portion of the nephridium (Fig. 11, *N*) is much larger than in the female, and the glandular part (*G*) appears less massive. In the sections of a female whose cavity was filled with eggs in such a way as to indicate that ovulation had not occurred the tubular part of the nephridia was so compressed and displaced as to preclude the possibility that it could be used for the discharge of eggs, at least at the beginning of ovulation. In the male, on the contrary, there is abundant evidence that these organs are in an hypertrophied condition, and probably they function as vasa deferentia. They have large lumens, and protrude well into the middle of the body.

Genital Products.

As in other polychetes, the eggs and sperm are produced by proliferation from the coelomic peritoneum. They are found in all the full-sized segments of the body, beginning at about the 19th or 20th setigerous segment. Ripe eggs were found in sections two or three segments anterior to the union of gizzard and intestine. Figure 30 shows a cross-section of a female which has not deposited any of her eggs. In this case the eggs are so numerous that the segment is stretched until the body wall is very thin. The eggs occupy every available cavity. As has been indicated it appears to the writers that the ova could not use the nephridia in this case, but must escape by rupture of the body wall.

BIBLIOGRAPHY.

ANDREWS, A. E.

1892. On the Eyes of Polychaetous Annelids. Journal of Morphology, **7**:169–222, pl. 9–12.

BENHAM, W. B.

1901. Archiannelida, Polychaeta, and Myzostomaria. Cambridge Nat. Hist., **2**:239–344.

CLAPARÈDE, A. R. E.

1863. Beobachtungen über Anatomie und Entwicklungsgeschichte wirbelloser Theire, viii + 120 p., 18 pl. [pp. 38–48.]

EHLERS, E.

1864–68. Die Borstenwürmer. xx + 722 p., 24 Taf. [Erste Abt., 1864 pp. 203–232.]

GALLOWAY, T. W.

1908. A Case of Phosphorescence as a Mating Adaptation. School Sci. and Math. May, 1908. 5 pp.

HASWELL, W. A.

1886. On the Structure of the so-called Glandular Ventricle (Drüsenmagen) of Syllis. Quart. Jour. Micr. Sci., **26**:471–480, pl. 25.

1889. A Comparative Study of Striated Muscle. Quart. Jour. Micr. Sci., **30**:31–50, pl. 4, 5.

LANGERHANS, P.

1879. Die Wurmfauna von Madeira. Zeitschr. f. wiss. Zool., **32**:513–592, Taf. 31–33. [p. 553–556.]

MALAQUIN, ALPHONSE.

1893. Recherches sur les Syllidiens. Lille. 477 p., 13 pl.

McINTOSH, W. C.

1808–10. A Monograph of the British Annelids. Ray Society. **2**, viii + 524 p., pl. 43–70. [Syllidae, p. 137–248.]

MOORE, J. P.

1909. The Polychaeteous Annelids dredged by the U. S. S. "Albatross" off the Coast of Southern California in 1904. Proc. Phila. Acad. Nat. Sci., **61**:321–351, pl. 15, 16.

ST. JOSEPH, A. DE.

1886. Les Annélides Polychètes des Cotes de France. Ann. d. Sci. Nat., Zool., ser. 9, **3**:145–260, pl. 1–5.

VERRILL, A. E.

1900. Additions to the Turbellaria, Nemertina, and Annelida of the Bermudas with Revisions of some New England Genera and Species. Trans. Conn. Acad. Sci., **10**:595–698, pl. 70. [p. 627–8.]

EXPLANATION OF PLATES

Drawings were made from female specimens, except where the contrary is indicated.

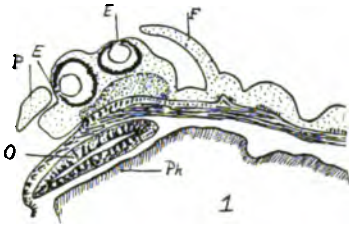
Plate 1

Fig. 1. A para-sagittal section of the dorsal part of the head, diagrammatic. *E*, the pupils of the eyes (see also Fig. 7); *F*, flap of peristomium (see also Fig. 6); *O*, a glandular fold at the dorsal anterior edge of the pharynx (see also Fig. 2); *P*, palp; *Ph*, pharynx.

Fig. 2. A sagittal section of the head and anterior segments, with the introvert withdrawn. *1*, the last peristomial (first setigerous) segment; *an*, an annular organ in the cardiac opening of the gizzard, consisting of modified epithelium and circular muscle fibres; *Br*, brain; *Cu*, cuticula of the esophagus (*E*); *F*, a flap projecting forward from the 3rd peristomial segment (see also Figs. 1 and 6); *G*, gizzard, in which the dorsal (*D*) and ventral (*V*) walls are cut in different relation to the elements composing the wall; *M*, mouth; *O*, a thin-walled glandular pocket at the anterior, dorsal edge of the pharynx; *P*, palp; *Ph*, pharynx, with thick muscular wall; *Ph'*, thin-walled, flexible portion of pharynx; *t*, cuticular teeth in anterior margin of esophagus. × 40.

Fig. 3. A frontal section through head and anterior segments, with the introvert protruded. Lettering as in Fig. 2. × 40.

Fig. 4. Longitudinal section at junction of gizzard and intestine. *G*, gizzard; *I*, intestine; *S*, sphincter muscle at beginning of intestine; *V*, valvular enlargement at anterior end of intestine. × 40.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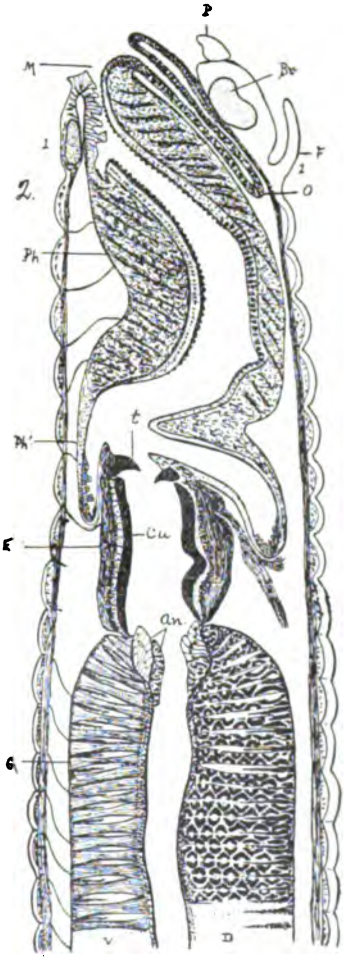
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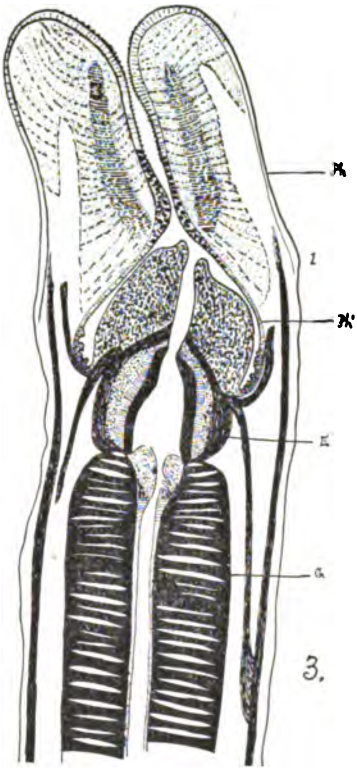
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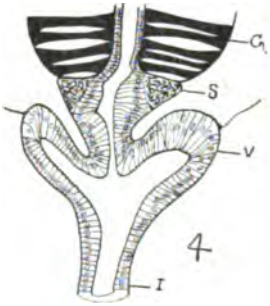
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Plate 2

Fig. 5. Ventral view of the head; *l*, lateral tentacle of the prostomium; *m*, median tentacle; *P*, palpus; 1, 2, 3, the peristomial tentacles or cirri, The lower lip is shown to be an anterior projection of the first setigerous segment. × 15.

Fig. 6. Longitudinal section through dorsal body-wall immediately in front of 3rd peristomial (first setigerous) segment, B, brain; E, epithelium; *E′*, the thickened sensory epithelium in front of the peristomial flap (*F*). × 300.

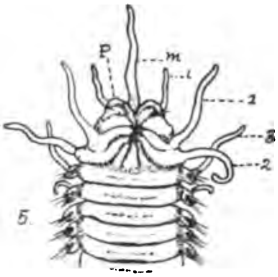
Fig. 7. Eye. A median section of anterior eye through pupil. *Cu*, cuticula; *E*, epithelium; *Le*, lens; *o. n.*, optic nerve; *Pe*, pedicel of the lens; *Pg*, pigment layer of the retina; *r*, rods, or layer formed by inner ends of retinal cells; *R*, retinal cells, deeper portion containing nuclei. × 330.

Fig. 8. Ommatidium. *C*, the nucleated part of the cell; *F*, fibre; *Pg*, pigment, which collectively makes the pigment cup of the eye; *r*, the rod or inner end of cell. × 500.

Fig. 9. Setae-sac of dorsal bristles, cut in the long axis of the setae. *A*, aciculum; *c*, bristle cell, from which the bristle arises; *m*, basement membrane; *s*, seta (see also Fig. 16). × 125.

Fig. 10. Cross-section of two parapodia showing both the dorsal and ventral bristle sacs cut across the long axis of the setae. *A*, acicula (the smaller light ovals are the setae); *Ci*, dorsal cirrus; *n*, nephridium; *S′*, ventral seta-sac; *S″*, dorsal sac, with the swimming bristles in two parallel rows. The dots and dashes are muscular elements. × 50.

Fig. 11. Cross-section of a segment in the mid-region of the body of a male. *G*, glandular part of nephridium; *N*, the tubular portion of nephridium, much enlarged and used as sperm-ducts; *T*, the twisted gland cells (see Fig. 22), numerous in the dorsal body wall. × 50.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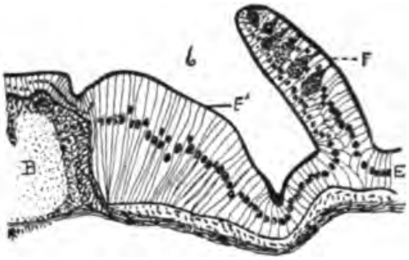
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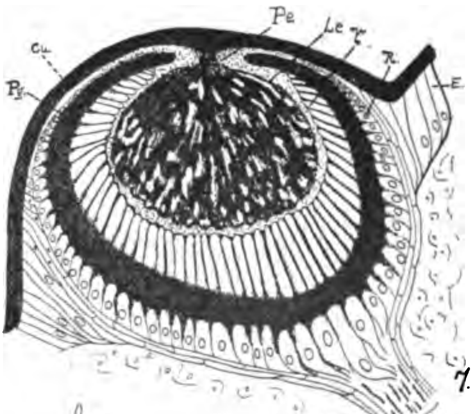
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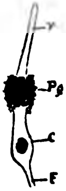
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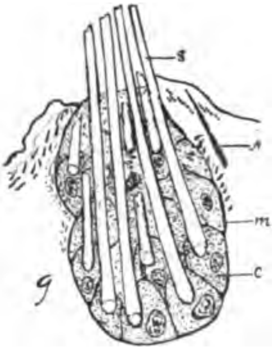
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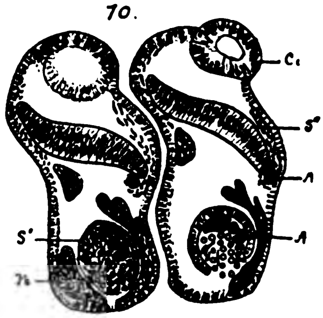
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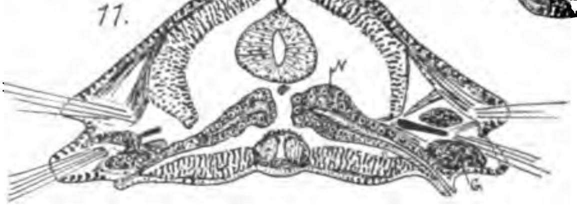
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Plate 3

Fig. 12. Dorsal view of head and anterior body segments. *Ey*, eyes, occurring in pairs on flaps; *L*, lateral tentacles of the prostomium; *M*, median tentacle; *P*, palpus; *Ph*, introvert protruded; 1, 2, 3, the tentacles of the three peristomial segments. × 15.

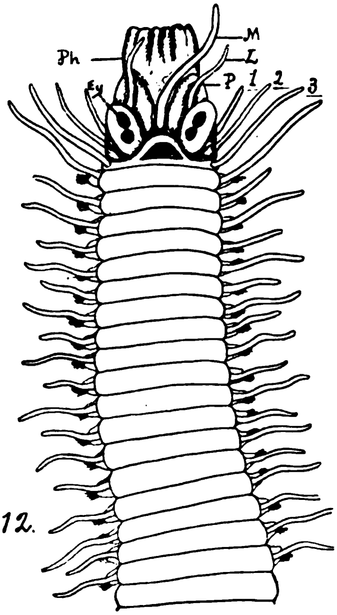
Fig. 13. Lateral view of the head. Lettering as in Fig. 12. × 15.

Fig. 14. Sagittal section through buccal cavity, showing beginning of the pharynx. *D*, dorsal; *V*, ventral; *dt*, conical denticles of cuticula in wall of pharynx; *g*, a patch of glandular cells in floor of mouth; *Ph*, lumen of pharynx.

Fig. 15. Gland cells of epidermis. *C*, cuticula; *E*, epithelium; *g*, gland cell showing characteristic inner reticulated protoplasm and outer striated protoplasm. × 600.

Fig. 16. Setae. *D*, the dorsal, capilliform, swimming setae of the mid-body region; *V*, the ventral jointed setae, occurring the whole length of the body.

Fig. 17. Cross-section of the body in the mid-region of body. *Ci*, dorsal cirrus; *I*, lumen of intestine; *O*, ova in body cavity. × 50.



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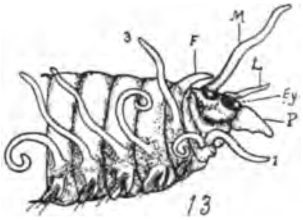

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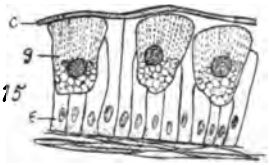

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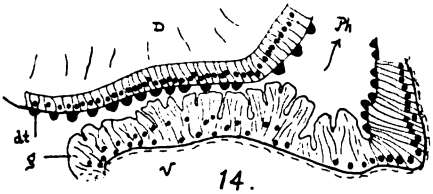

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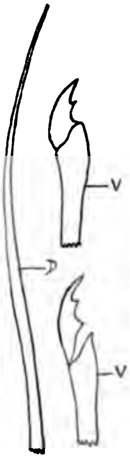

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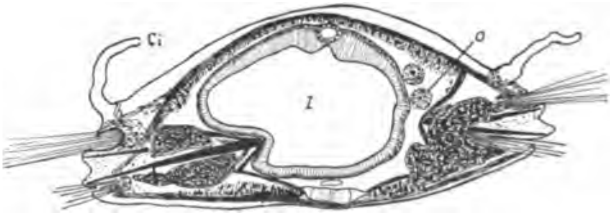


Plate 4

Fig. 18. Cross-section of pharynx and esophagus, with introvert withdrawn. *E*, muscular wall of the esophagus, showing the lateral mass of muscular fibres connected with the tooth apparatus; *ep*, epithelial layer that secretes the ventral teeth; *Df*, a longitudinal, dorsal fold of the pharynx that invades the front of the esophagus in introversion; *Ph*, muscular wall of the pharynx. × 65.

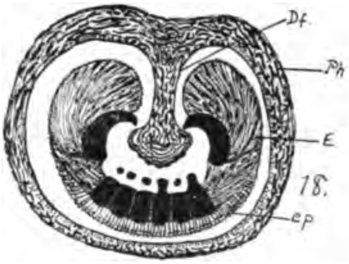
Fig. 19. Similar cross-section a little anterior to Fig. 18. × 65.

Fig. 20. Cross-section in mid-region of body, including a dissepiment (*Di*). *1*, dorsal ramus of notopodium, bearing dorsal cirrus (*Ci*); *2*, ventral ramus of notopodium, bearing swimming bristles supported by aciculum; *3*, dorsal ramus of neuropodium, bearing bristles and acicula; *4*, ventral ramus of neuropodium with no appendages. ×50.

Fig. 21. Cross-section of body through the gizzard. *A*, aciculum; *Ci*, dorsal cirrus; m^1, layer of circular muscles of body wall; m^2, longitudinal muscles; *Ra*, raphe where there is an anastomosis of the fibres of the annular band of muscle fibres (2) with the innermost layer of the gizzard. The numerals indicate three successive zones of elements in the wall: *1*, the peritoneum and thin outer muscle layer; *2*, annular bands of fibres, much flattened in planes parallel with the long axis of the animal suggested by the broken lines; *3*, muscular columns which make up the bulk of the wall of the organ. Within this is a thin layer consisting of muscles, epithelium, and cuticula. × 65.

Fig. 22. "Twisted" glands (phosphorescent?) from epidermis of female. *Po*, pore in cuticula (*c*); *Sg*, the gland with constrictions. × 680.

Fig. 22a. Similar glands in the dorsal epithelium of a male specimen. × 680.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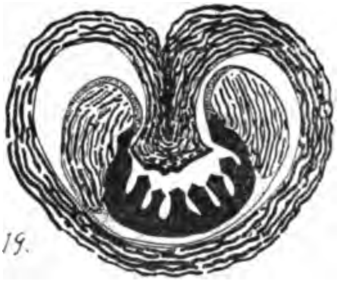
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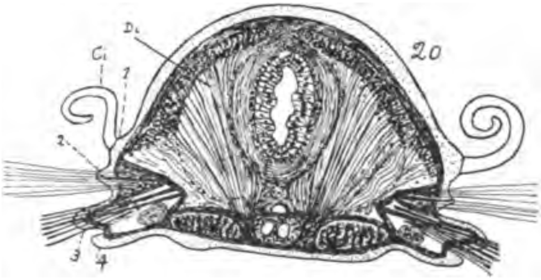
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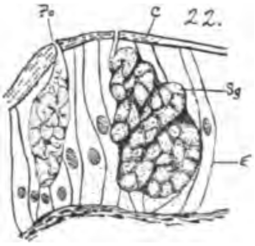
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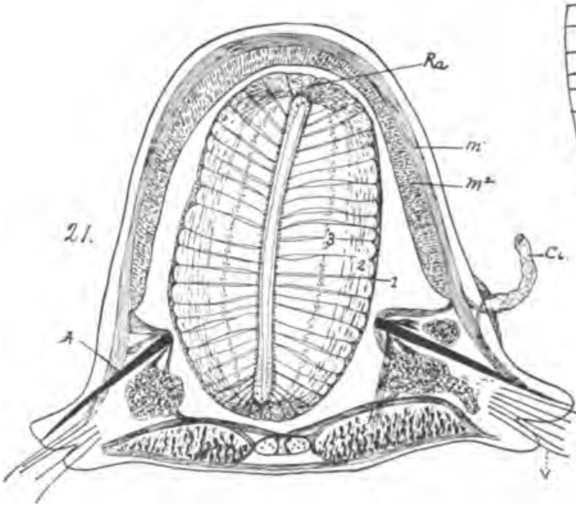
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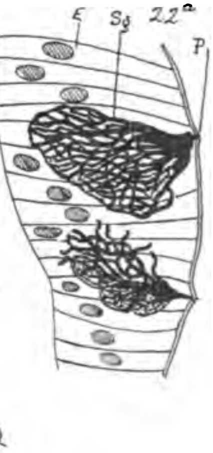
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Plate 5

Fig. 23. Detail of ventral floor of esophagus, longitudinal section, in region of teeth. *Cu*, cuticula lining esophagus; *Ep*, epithelial layer; *g*, glandular epithelium in the thin portion of pharynx; *t*, one of the ventral teeth.

Fig. 24. Two muscle columns of the gizzard, taken from transverse section of that organ (see Fig. 21). Lettering in figures 24–28: *cb*, "circular band," a thin sheet of muscle whose fibres encircle the organ; *col*, the radial muscular columns, whose fibres radiate from the lumen; *f*, a fissure separating the halves of one column; *mi*, thin inner muscular layer; *mo*, a thin outer muscular layer; *n*, nucleated protoplasm between the outer ends of the half-columns.

Fig. 25. Section perpendicular to 24, transverse to the columns and tangential to gizzard, just as near the outer surface as possible. Anterior is up in the Fig. × 320.

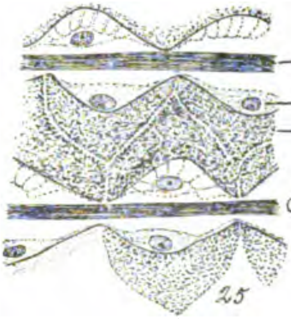
Fig. 26. Section transverse to the columns (parallel with 25), at a deeper level. × 160.

Fig. 27. Section parallel with 24 and 25, transverse to the columns at their inner ends. × 160.

Fig. 28. Longitudinal section of columns (outer end) at right angle to Fig. 24, showing them as they appear in longitudinal section of the gizzard. × 160.

Fig. 29. Dorsal wall of intestinal tract showing relation of dorsal blood vessel to it. *cil*, cilia; *d*, dorsal blood vessel; *Ep*, epithelial lining of digestive tract; *L*, lumen of blood vessel. × 360.

Fig. 30. Cross-section from mid-body region of a female in which ovulation has not commenced. *N*, glandular part of nephridium; *O*, ova; *W*, the much stretched and thinned dermo-muscular wall. × 40.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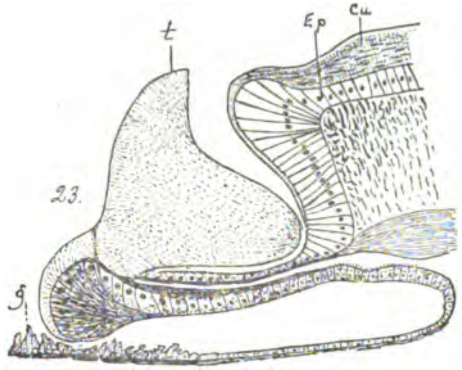
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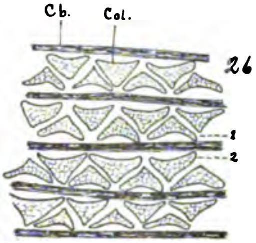
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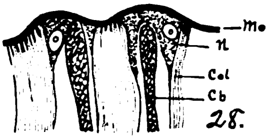
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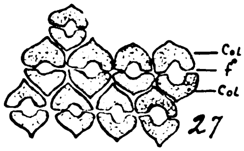
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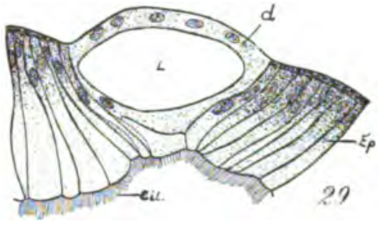
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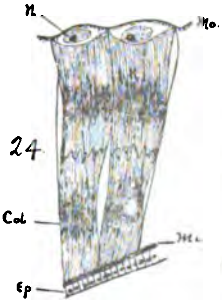
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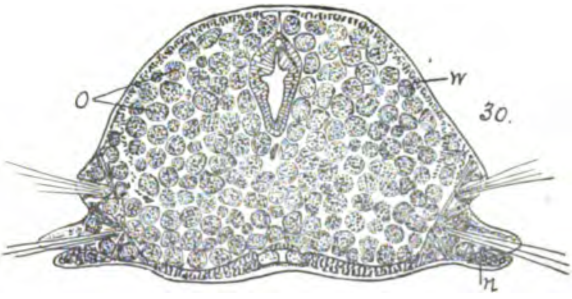
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Vol. 2.

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THE TASTE OF ACIDS[1]

W. J. CROZIER

TWO FIGURES

I. The problem of irritability is essentially a matter concerning sense organs; in these structures we find cells "as nearly as possible unifunctional" (Lucas, '09, p. 328) with respect to the capacity for being stimulated. Hence the analysis of irritability should properly be founded upon the study of receptor physiology in higher animals, rather than upon an examination of the properties of protoplasm in deceptively simple protozoans.

Two contrasted methods have in the past been used in the study of stimulation. The first of these deals with the responses of unicellular organisms, together with the (usually vague) application of principles derived from cells of more or less generalized type. The second method has consisted in the analysis of sensations. Yet, if we consider such questions as those involved in taste stimulation, we find that no definite conclusions have been reached. The very complexity of organization in 'simple' forms is probably in the main responsible for the results obtained in the first field, results which we find stated in such weak generalizations as occur in Verworn's book ('13, pp. 41, 86, 94). The second procedure, the study of human sense organs, has inevitably come to be clouded by psychological interpretations.

It appears that the possibility of arriving at some understanding of what occurs in stimulation has not yet been sufficiently tested out from the standpoint of sense organ irritability as revealed in animal reactions. This paper is a contribution toward that end; it deals with the problem presented by the taste of acids. I am indebted to Prof. G. H. Parker for his criticism of the manuscript.

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[1] Contributions from the Bermuda Biological Station for Research, No. 46.

II. Practically the whole of the difficulty is contained in the often considered case of acetic and hydrochloric acids. A comparative study of the reactions to these and other acids as given by the earthworm (Hurwitz, '10; Crozier, '16), leads to a preliminary simplification of the matter, namely to the formulation of two separate problems: these acids stimulate, they also result in a sour taste. These two problems must be considered independently; we must distinguish between (a) that property of an acid which causes it to be efficient in stimulation and (b) that which determines the sourness of an acid solution.

The relative efficiency of an acid as an agent of stimulation may be measured by the liminal effective dilution. The point I wish to make is that the property of hydrochloric acid which causes it to be tasted in solutions about five times as dilute as that limiting the tastability of acetic acid may have no immediate connection with the common cause of the sourness perceived when these acids act upon the tongue.

This point of view contains the possibility of an explanation for (and is, reciprocally, in part justified by) the fact that acids exhibit a characteristic astringency in solutions so dilute that they are no longer sour. There is some evidence, also, that the sourness and astringency may be separated experimentally, as by the use of cocaine. There is therefore ground for the opinion that in taste excitation by acids two processes occur, and that the production of a sour taste is the secondary one.

The points which require solution with respect to human taste excitation by acetic and hydrochloric acids are:

(a) Both these substances stimulate.

(b) They stimulate in different degrees, in dilute solutions the stimulus from hydrochloric acid being the more intense.

(c) They both result in a sour taste.

(d) They fail to stimulate when too greatly diluted.

(e) The dilutions which limit the capacity of the two acids to stimulate are very different (HCl n/900 ±; acetic, n/200 ±).

Each of these points—except (c)—is closely paralleled by the details of earthworm reactions to acids (Crozier, '16), and also by the reactions of Æolosoma, a freshwater oligochaet (Kribs, '10).

It adds somewhat to the clearness of the discussion if, as I have previously proposed (Crozier, '14, p. 16), we restrict the word 'stimulus' to mean the change induced in a receptor by the action of a stimulating agent. The explanation of the acid taste is made easier by the fact that one is not called upon to account for a sour taste resulting from heterologous stimulation; there is no good evidence for the existence of a sour taste not directly produced by acid. The extreme specialization of the acid taste makes it a very favorable case for analysis.

III. It is sufficiently obvious that only the surface of the receptor is immediately concerned in stimulation. This view is reasonable upon purely morphological grounds, such as the modifications of the exposed ends of sensory cells[2] and the relations of nerve fibrils to the surface of secondary sense cells. The way in which this conception of the cell surface as the organ of irritability has been elaborated by R. S. Lillie and others need not be discussed here. But it may be pointed out that the method of interpreting mechanical excitation has been indicated by Osterhout ('15) and by such observations as that of Evans and Winternitz (Evans and Schulemann, '14, p. 453). Unequivocal evidence in this direction is afforded by Harvey's work on the penetration of cells by alkalies (Harvey, '14 c), by his experiments with acids (Harvey, '14 c), and by my own study of this latter subject (Crozier, '15, '16); the evidence referred to is, in brief, to the effect that penetration of the body of the cell substance is altogether too slow a process to account for the rapidity of taste stimulation. The slowness of acid penetration is due to the resistance offered by the cell surface, a resistance which practically disappears with the death of the cell. An examination of cell penetration by various acids, employing a wide range of dilutions, should therefore yield valuable information regarding the composition and behavior of the resistant cell surface. In several cases a study of this kind has been made possible by the discovery of intracellular indicators sensitive toward acids (Harvey, '14 b, Crozier, '15).

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[2] The propable chemoreceptors of the earthworm have been described by Miss Langdon ('95) and Bovard ('04).

Measurements of cell penetration by acids show, so far as concerns the present problem: 1) that both acetic and hydrochloric acids are able to penetrate the cell; 2) other things being

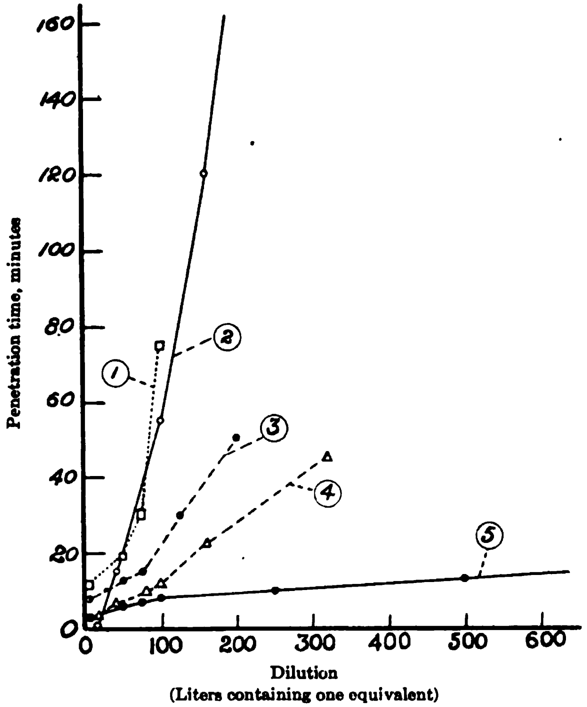

Fig. 1 The average time of penetration of cells by hydrochloric, butyric, and acetic acids.

1, Acetic acid
3, Butyric acid } integument of Chromodoris zebra, a nudibranch.
5, Hydrochloric acid
2, Butyric acid } testis epithelium of Stichopus ananus, a holothurian.
4. Hydrocloric acid
1, *3*, *5*, from Crozier ('16), at 27°.
2, *4*, from Harvey ('14 c), at 28°.

Butyric acid is introduced for comparison, since Harvey (loc. cit.) gives no data for dilutions of acetic; the principle is the same for both these acids. It should be noted that in Harvey's experiments the acid was dissolved in a balanced salt solution, in mine in distilled water.

equal, the speed of this penetration varies with the acid and its concentration; 3) the curves relating penetration time to concentration are different for the two acids, dilute solutions of hydrochloric penetrating much more easily than acetic. Points of resemblance, in fact of identity, are found among the characteristics of stimulation by these acids, and have been enumerated as a, b, e, in section II. The closeness of the parallelism indicates that the stimulus is due to the penetration of the surface layer of the cell. In stimulation by these acids the essential step is the union of the stimulating agent with some constituent of the ceptor surface.

According to Becker und Herzog ('07), the decreasing order of intensity of taste sensation due to the acids they tested was HCl, HNO_3, trichloroacetic, formic, lactic, acetic, butyric. These acids penetrate the indicator-containing cells of Chromodoris zebra in the order HCl, HNO_3, formic, monochloroacetic, lactic, butyric, acetic (considering a range of concentrations; Crozier, '16). There is reason to believe that trichloroacetic penetrates more speedily than monochloroacetic; and with this in mind, the parallelism between these two series becomes surprisingly close. This particular case is quoted with the purpose of pointing out that a small series of acids, such as that just cited, may to all appearances support the view that the intensity of taste excitation is proportional to the ionization strength of the acids; similarly, a penetration series may be chosen which seems to prove a corresponding relation. Yet, further examination of a large number of acids shows that ionization is but one of the factors determining cell penetrating power, and the existence of influences in addition to ionization has also been suspected with reference to acid stimulation; direct evidence upon this point has hitherto been lacking.

IV. Analysis of penetration data obtained with eighteen acids has shown (Crozier, '16) that in any given case ability to penetrate the cell appears to depend upon two (or more) factors: one is the ionization strength of the acid; the second depends upon some other aspect of the acid molecule. To account for these results it is further necessary to assume that the cell surface is of heterogeneous constitution. This conclusion may be extended to include the surface of chemoreceptors, since it enables us to account not only for the reactions of the earthworm to dilutions of acids (Crozier, '16), but also clears up a well known anomaly in regard to human taste.

It was shown by Richards ('98) and by Kahlenberg ('98) that the hydrogen ion must in some way be responsible for the sour taste. Yet acetic acid, which ceases to be tasted at dilutions

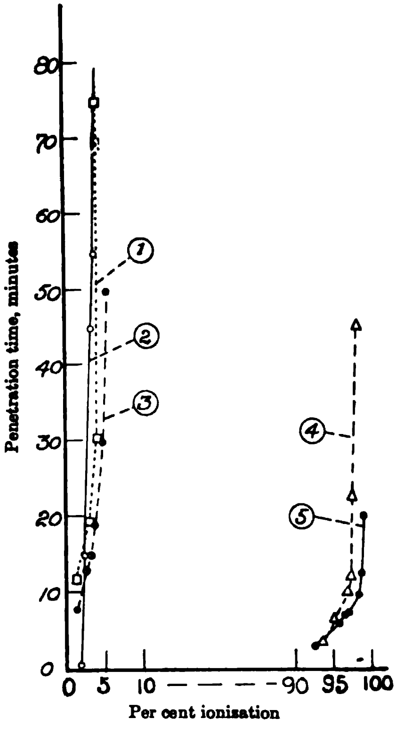

Fig. 2 The relation between percentage ionization and time of cell penetration from solutions of hydrochloric, butyric, and acetic acids.

1, Acetic acid
3, Butyric acid } integument of Chromodoris zebra.
5, Hydrochloric acid

2, Butyric acid } testis epithelium of Stichopus ananus.
4, Hydrochloric acid

1, *3*, *5*, from Crozier ('16).
2. *4*. from Harvey ('14c).

below about n/200 is only 6 per cent dissociated at that concentration, whereas hydrochloric acid, fully dissociated (99 per cent), is tasted down to about n/900. The actual hydrogen ion concentrations in these solutions limiting tastability are: HCl (n/900), $C_H = 0.00119$ N; acetic (n/200), $C_H = 0.00035$ N. Acetic acid is more stimulating than would be calculated from its dissociation.

Exactly similar conditions are found in studying the penetration of cells by acids, as will be clear from an inspection of the two sets of curves in figure 2. In table 1 acid solutions giving the same penetration time appear in the same horizontal row; it is seen that the penetration of cells by acetic acid is much more efficient than if its dissociation were the deciding influence.

TABLE 1

The ionization of hydrochloric, acetic, and butyric acids, in solutions which penetrate cells within equal times. For sources of data see corresponding reference numbers in figure 2.

TIME OF PENETRATION, MINUTES	IONIZATION, PER CENT				
	Hydrochloric		Acetic	Butyric	
	[5]	[4]	[1]	[3]	[2]
5	95.0	94.5			2.0
10	98.5	96.7		2.0	2.1
15	98.8	97.4	2.1	3.3	2.5
20	99.0	97.5	3.0	4.0	2.6
50			4.0	5.5	3.5

It follows that the stimulus produced by acetic acid is due, in the first place, to its union with the cell surface; this penetration of the plasma membrane is more efficient than if dissociation strength were the determining factor. The indication is that this acid dissolves in a fatty substance located at the cell surface (for further evidence, see Crozier, '16). The stimulus due to hydrochloric acid also depends upon its penetration of the cell surface, but there is some evidence that it involves proteins.

It is not clear to what extent stimulation by acetic and hydrochloric acids may be interpreted in terms of the effects which these substances have upon surface colloidal conditions, and thus upon cell permeability (Spaeth, '16). Herlitzka ('10) has discussed this question with reference to the taste of salts, but measurements of the effect of acid on permeability (Osterhout, '14) have not considered the possibility that characteristically different results may follow from the action of diverse acids; Osterhout's ('14) measurements deal only with hydrochloric acid.

V. There remains to be accounted for the production of a unitary taste quality, the sourness of acid solutions. This must be related to ionizable hydrogen (Kahlenberg, '98, Richards, '98). But it has just been shown that hydrogen ions outside the cell surface cannot be the effective agents, since stimulation by these acids parallels so closely the peculiarities of their penetration of the cell. The hydrogen ion must therefore act after the acid has united with the ceptor surface. At least two possibilities are obvious: (1) the presence of potentially ionizable hydrogen within sufficiently concentrated undissociated molecules is enough to produce the sour taste, or (2) the acids ionize secondarily after entering. The first suggestion is not so far fetched as it may at first seem, though it is not in accord with current teaching. Numerous parallels could be drawn from the taste of organic substances (Francis and Fortescue-Brickdale, '08, pp. 331 et seq.). I believe that this view is possibly correct, although something could also be said for the hypothesis of secondary ionization following some reaction involved in gaining admission to the outer layer of the cell. It is in this latter direction that the explanation (Beutner, '14) must be sought for the source of electromotive effects accompanying stimulation in general, which presumably occur also in taste excitation.[3] This interpretation disposes of the difficulty (very conspicuous in the case of taste) which is encountered by those who would make the process of stimulation and the production of electromotive effects completely identical in every case.

[3] A taste cell bathed by a stimulating solution is in a condition very similar indeed to that of the various tissues experimented upon by Loeb and Beutner (Loeb, '15).

SUMMARY

An attempt has been made to account for the action of acetic and hydrochloric acids upon the sense of taste. It is shown that the stimulus due to these substances probably depends upon their union with diverse constituents of the ceptor surface. It is thus possible to explain, by comparison with the penetration of cells by these acids: how they stimulate at all, why they stimulate to different degrees, and why acetic acid gives a more powerful stimulus than if it acted by dissociation alone. Why these acids produce a unitary (sour) taste quality is a problem of a different order; it is possible that this depends upon the presence of potentially ionizable hydrogen within undissociated acid molecules, though secondary ionization may also play a part.

AGAR'S ISLAND
BERMUDA.

LITERATURE CITED

Becker, C. T. und Herzog, R. O. 1907 Zur Kenntnis des Geschmackes. I. Mitteilung. Zeits. f. physiol. Chem., Bd. 52, p. 496–505.

Beutner, R. 1914 Studies on a new kind of E. M. F. Jour. Amer. Chem. Soc., vol. 36, p. 2040–2045.

Bovard, J. F. 1904 The distribution of the sense organs in Microscolex elegans. Univ. Calif. Publ., Zoöl., vol. 1, p. 269–286.

Crozier, W. J. 1914 The orientation of a holothurian by light. Amer. Jour. Physiol., vol. 36, p. 8–20.

1915 On cell penetration by acids. Science, N. S., vol. 42, p. 735–736.

1916 Cell penetration by acids. Jour. Biol. Chem., vol. 24, p. 255–279.

Evans, H. M., and Schulemann, W. 1914 The action of vital stains belonging to the benzidine group. Science, N. S., vol. 39, p. 443–454.

Francis, F., and Fortescue-Brickdale, J. M. 1908 The chemical basis of pharmacology. London, 8°, xii + 372 pp.

Harvey, E. N. 1914 a The relation between the rate of penetration of marine tissues by alkali and the change in functional activity induced by the alkali. Publ. Carnegie Instn. Wash., No. 183, p. 131–146.

1914 b Cell permeability for acids. Science, N. S., vol. 39, p. 947–949.

1914 c The permeability of cells for acids. Intern. Zeits. Physik.-chem. Biol., Bd. 1, p. 463–478.

Herlitzka, A. 1910 Contributo all'analisi fisico-chimica del sapore dei sali. Archivo d. fisol., vol. 7, p. 557–578.

Hurwitz, S. H. 1910 The reactions of earthworms to acids. Proc. Amer. Acad. Arts and Sci., vol. 44, p. 67–81.

KAHLENBERG, L. 1898 The action of solutions on the sense of taste. Bull. Univ. Wisconsin, Sci. Ser., vol. 2, p. 1–31.

KRIBS, H. G. 1910 The reactions of Æolosoma to chemical stimuli. Jour. Exp. Zoöl., vol. 8, p. 43–74.

LANGDON, FANNY 1895 The sense-organs of Lumbricus agricola, Hoffm., Jour. Morph., vol. 11, p. 193–234, pl. 13.

LARGUIER DES BANCELS, J. 1912 Le goût et l'odorat. Paris, 8°, vii + 94 pp.

LOEB, J. 1915 Electromotive phenomena and membrane permeability. Science, N. S., vol. 42, p. 643–646.

LUCAS, K. 1909 The evolution of animal function, II. Science Progress, vol. 3, p. 321–331.

OSTERHOUT, W. J. V. 1914 The effect of acid on permeability. Jour. Biol. Chem., vol. 19, p. 493–501.

1915 The nature of mechanical stimulation. Science, N. S., vol. 41, p. 175.

RICHARDS, T. W. 1898 The relation of the taste of acids to their degree of dissociation. Amer. Chem. Jour., vol. 20, p. 121–126.

SPAETH, R. A. 1916 The vital equilibrium. Science, N. S., vol. 43, p. 502–509.

VERWORN, M. 1913 Irritability. New Haven, 8°, xii + 264 pp.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

VOL. 1.

1. **COLE, L. J.** — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. **GULICK, A.** — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. **BOWDITCH, H.** — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. **COE, W. R.** — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. **BIGELOW, H. B.** — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
6. **MARK, E. L.** — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **BARBOUR, T.** — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
8. **BLACKMAN, M. W.** — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–343, 1 pl. Sept., 1905.
9. **CONGDON, E. D.** — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. **BLACKMAN, M. W.** — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pls. Feb., 1907.

VOL. 2.

11. **LINTON, E.** — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. **PARKER, G. H.** — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16) : 413–455. Apr., 1908.
13. **COLE, J. F.** — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) ; 49–56, 1 chart. June [Aug.], 1908.
14. **MORGULIS, S.** — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
15. **MORGULIS, S.** — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
16. **RAND, H. W.** — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2) : 189–238, 2 pls. Sept., 1909.
17. **MARK, E. L.** — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate : 6 pp. Nov., 1909.
18. **SMALLWOOD, W. M.** — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1) : 137–145. June, 1910.
19. **SMALLWOOD, W. M., AND ROGERS, C. G.** — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
20. **CARPENTER, F. W.** — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6) : 147–162, 1 pl. Oct., 1910.
21. **ESTERLY, C. O.** — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7) : 217–226, 4 pls. July, 1911.
22. **COWDRY, E. V.** — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10** : 1–53, 4 pl. July, 1911.
23. **GROSS, A. O.** — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. **SMALLWOOD, W. M.** — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773, 4 pls. May, 1913.
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29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
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35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. Crozier, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. Crozier, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.
41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
42. Hecht, S. — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3) : 429–434. Apr., 1916.
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44. Crozier, W. J. — Some Indicators from Animal Tissues. Jour. Biol. Chem., **24** (4) : 443–445. Apr., 1916.
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35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. Mayor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. Crozier, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
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41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
42. Hecht, S. — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3) : 429–434. Apr., 1916.
43. Crozier, W. J. — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3) : 297–356. Apr., 1916.
44. Crozier, W. J. — Some Indicators from Animal Tissues. Jour. Biol. Chem., **24** (4) : 443–445. Apr., 1916.
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No. 47.

NOTES ON THE REACTIONS OF BIVALVE MOLLUSKS TO CHANGES IN LIGHT INTENSITY: IMAGE FORMATION IN PECTEN.

BY D. H. WENRICH.

FROM THE JOURNAL OF ANIMAL BEHAVIOR, VOL. VI, NO. 4.

CAMBRIDGE, MASS., U. S. A.
AUGUST, 1916.

NOTES ON THE REACTIONS OF BIVALVE MOLLUSKS TO CHANGES IN LIGHT INTENSITY: IMAGE FORMATION IN PECTEN

D. H. WENRICH

Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College. No. 277

Contributions from the Bermuda Biological Station for Research. No. 47

PAGE
I. Introduction 297
II. Results with Anadonta 298
  A. Methods 298
  B. Results 299
    1. Kind and degree of sensitivity 300
    2. Variations in sensitivity 300
      a. Variations in a single individual 300
      b. Variations among different individuals 303
    3. Comparative measurements of sensitivity 303
III. Results with Marine Species 304
  A. Qualitative tests with different species 305
    1. Classification based on sensitivity 305
    2. Relation of sensitivity to presence or absence of pigment 306
    3. Differences in reactions to increase and decrease of light intensity 306
  B. Results with Pecten 307
    1. Sensitivity to increase and decrease 307
      a. Methods 307
      b. Results 308
    2. Reactions toward a moving object 309
      a. Experiments 309
      b. Discussion 311
IV. Summary 316
V. List of Papers Cited 317

I. INTRODUCTION

During the summer of 1913, while enjoying the privileges of the Bermuda Biological Station for Research, the writer became interested in the reactions of bivalve mollusks to changes in light intensity. Little more was done, however, than to observe the constancy with which the various species tested responded to shadows, that is, to decreases in the intensity of the light. Further observations were made in the Harvard Zoölogical Laboratory during the first half of the 1913-14 school year, when a few hours each week were devoted to a study of the reactions to changes in light intensity of one of the common fresh-water mussels, Anodonta fluviatilis.[1] I am indebted to Professor G. H. Parker for advice and assistance during this part of the work. Observations on marine forms were renewed the following summer while the writer was occupying a table at the U. S. Fisheries Laboratory at Woods Hole. Here special attention was given to the reactions of Pecten to moving objects.

II. RESULTS WITH ANODONTA

A. Methods

In the experiments on this fresh-water mussel, the following arrangements were made use of for the control of the lights and for making the observations. The vessel in which the animals were placed for experimental study was a rectangular museum jar of a capacity of about 6 liters. Coarse sand was placed in the bottom to a depth of about 5 centimeters, and enough water was added to make a depth of from 10 to 15 centimeters above the sand. The mussels were always placed near one end of the jar with their siphons toward that end, and with their long axes parallel to the longer horizontal axis of the jar. A wooden box was painted black inside and provided with a black cover through which a small observation hole was made. In one end of the box another hole, 5 centimeters in diameter, was bored about 7 centimeters from the bottom, so that its height would correspond to that of the siphons of the mussels when the jar containing them was placed in the box.

Electric lights (incandescent bulbs) were employed as sources of illumination. Two sources were placed on opposite sides of a line which coincided with the long axis of the mussels in the jar and the center of the hole in the end of the box. The lights were so placed that their rays made equal angles with this line. A screen was arranged so that the light from one of the bulbs could be suddenly cut off without interfering with the rays from the other bulb. The screen was not allowed to set up vibrations by touching the floor. Further precautions against vibrations, to which the animals are very sensitive, were taken by supporting the experimentation-box by another box on the cement floor of a basement room, and then placing wads of paper under the lower box, between the two boxes, and under the jar containing the animals. All other light than that used in experimentation was, of course, excluded from the room.

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[1] I am indebted to Mr. W. F. Clapp for advice in the determination of the species mentioned in this paper.

In order to have an expression indicating the intensities of the different lights and their relationships the following formula was employed: Let A equal the intensity of the light—expressed in candle-meters—cut off to produce the decrease; let B equal the intensity of the uninterrupted light—expressed in candle-meters. Then A + B would equal the total intensity before decrease, and $\frac{A}{A + B}$ would be the ratio of the amount of decrease to the total or maximum intensity. For a criterion of equal effects with different intensities, the " inhibition point " was determined; in other words, that value of $\frac{A}{A + B}$ for which A was the lowest intensity at which a response could be obtained when it was cut off. At a slightly lower value for A, B would be great enough, with respect to A, to inhibit a reaction when A was cut off. For the determination of this inhibition point, either A or B could be kept constant and the intensity of the other varied. The value of $\frac{A}{A + B}$ at the inhibition point was used as an index of the degree of sensitivity of an animal.

One source of possible error was the use of the commercial values for the candle-power of the light bulbs employed. The actual intensity of the light falling on the siphons was of course less than this because it had to pass through the glass of the jar and about 2 or 3 centimeters of water. No claim to complete accuracy can be made, therefore, but since the conditions were similar for all the experiments, it is believed that the results are accurate enough to be of value.

B. Results

The points determined in this investigation on Anodonta were, briefly: (1) The degree and kind of sensitivity; (2) the variations in sensitivity among individuals and in the same individual; (3) the recognition of a normal condition of sensitivity, during which could be obtained measurements useful in making comparisons.

1. Kind and Degree of Sensitivity

As a general rule the animals reacted by closing one or both siphons, seldom by closing the valves. The use of greater intensities of light might have resulted in greater muscular contraction in the reactions. The exhalant siphon was far more sensitive than the inhalant siphon and frequently was the only part that reacted to the stimuli. In all the experiments not a single instance was found in which a typical reaction was given in response to an increase in light intensity. On the other hand, decreases so slight as not to be detected by the eye of the experimenter gave rise to unquestionable and typical reactions.

2. Variations in Sensitivity

a. Variations in a Single Individual

The differences in the sensitivity of an animal at different times may be said to be due to differences in the "physiological states" of the animal. In attempting to account for these differences the following factors need to be considered: (1) The previous adaptation to light or darkness; (2) the previous condition with respect to stimulation or non-stimulation; (3) the previous condition with respect to a natural or unnatural position of the body of the animal; (4) the condition of the mantle chamber with respect to the presence or absence of a quantity of foreign material; and (5), probably, the presence or absence of eggs or embryos in the gills of the females.

(1) Only one experiment was carried out to test the differences in sensitivity due to the previous adaptation to light or darkness. In experiment No. 41, mussel No. 203 was left in the experimental box in the dark from 8:15 a. m. until 1:30 p. m., when a series of trials was made in rapid succession to determine the inhibition point. With the light B remaining constantly at a value of 2 candle-meters, the intensity of light A, which was a 10 candle-power bulb, was gradually increased by moving it closer to the animal until it reached the value of 2.5 candle-meters, when a slight but definite reaction was obtained. In this case the value of $\frac{A}{A+B}$ at a point very near to the inhibition point was 0.444. The animal was then left in quiet for half an hour with the lights remaining as above,

that is, A equaling 2.5, and B equaling 2 candle-meters. After this adaptation to a small amount of light, the animal was again tested and barely gave a response when A had the value of 0.5 candle-meters, light B remaining constantly at a value of 2 candle-meters. Under these conditions the value of $\frac{A}{A+B}$ was 0.20. In other words, the sensitivity had increased. This result is just the opposite of that obtained by C. Hess ('10) with Psammobia. He found the ' dark adapted" animals much more sensitive to changes in the intensity of the light than the "light adapted" ones. But in the case of Psammobia the animals were sensitive to increases in light intensity instead of to decreases. In the case of both Psammobia and Anodonta, therefore, the animals became more sensitive after exposure to that condition of their surroundings which tended to be non-stimulating.

(2) During experiments involving successive stimulations with intervals of from one to several minutes, if not carried far enough to cause fatigue, the sensitivity of the animals seems to increase. This was shown in numerous instances, of which the following is a good example. In experiment No. 43, mussel No. 203 was left for 4 hours under a constant light intensity of 10 candle-meters. A few tests showed the value of $\frac{A}{A+B}$ at the inhibition point to be about 0.14, when B had the value of 2 candle-meters. (It is to be noted that the sensitivity in this case is greater than that of the same animal in experiment No. 41, described above, and that the greater sensitivity is correlated with an adaptation to a higher intensity of light.) At the end of 2 hours, during which time half-hour periods of experimentation were alternated with half-hour periods of rest, the sensitivity had risen to such an extent that the value of $\frac{A}{A+B}$ at the inhibition point was 0.04, when B had the value of 2 candle-meters. In view of the 4-hour exposure to a light intensity of 10 candle meters previous to the tests made, it seems most probable that the increase in sensitivity was due to conditions resulting from the stimulations in the subsequent period of 2 hours,

rather than to the possible effect of the two hours additional exposure to light.

The increase in sensitivity in this experiment was of considerable proportions, and its absolute value indicates a rather high degree of sensitivity in these supposedly sluggish animals, for only 4 per cent of the total light intensity needed to be cut off to produce a reaction.

The increase in sensitivity as a result of repeated stimulations is indicated in another way. When an animal is left undisturbed it will spontaneously (i.e., without any observed stimulation) close its siphons from time to time. After a period of stimulation, however, the frequency of the spontaneous contractions of the siphons becomes greatly increased, which increase, it seems reasonable to conclude, is an indication of increased irritability to whatever causes operate to induce such closure when the animals are undisturbed as far as the conditions of the light are concerned.

(3) Occasionally individuals refused to respond in a typical manner, or perhaps refused to respond at all, although at other times the same individuals reacted in a normal way. Since it was noticed that of the various animals taken from the aquarium for experimentation, some were found lying on their sides and others in a natural position, the idea suggested itself that perhaps an unnatural position of the body tended to interfere with normal physiological activities. Such a result would not be unexpected in view of the presence of equilibration organs, in the form of the so-called otoliths. A series of pairs of animals were tested, one member of each pair having been in a natural position, while the other had been lying on its side or on its dorsum for a period of several days. The animal which had been in a natural position reacted in nearly every case more normally than the one which had been in an unnatural position. In a few cases the differences between the members of a pair were not very great, so that some other causes must have been involved in interfering with normal behavior.

(4) At other times, during the course of an experiment, an animal would cease to give consistent reactions. In some of these cases the cause seemed to be the accumulation of foreign material in the mantle cavity. For after a period—usually short—of non-responsiveness, a sudden contraction of muscles

would cause the valves to close partially, and at the same time eject a stream of water from the exhalant siphon carrying with it a greater or less amount of debris from the mantle cavity. Such activities would recur at short intervals until the mantle chamber was apparently free from the foreign material. Then the responses would become normal again. The presence of the foreign material in the mantle chamber seemed to act as a counter irritant, inhibiting for the time being the reactions toward decreases in light intensity.

(5) Some of the largest animals proved to be responsive very slightly or not at all, to decreases in light intensity. They would keep their siphons open wide, with vigorous currents passing in and out of the openings, and fail to close them when the light intensity was reduced. These were gravid females, and their non-responsiveness may have been due to the physiological necessity of maintaining a constant flow of water along the embryo-filled gills.

b. Variations Among Different Animals

The differences in sensitivity among different individuals is perhaps largely to be explained likewise by differences in physiological states induced by the same factors that were found to be effective in single individuals. Doubtless other unrecognized factors are involved in both cases, and individual peculiarities among the clams are also to be expected. A single example will indicate some of the differences to be met with. Mussel No. 203, normally, after stimulation at one-minute intervals for 15 or 20 minutes, begins to show irregularities in the responses, doubtless due to fatigue, and these irregularities increase if the stimulation is continued. In one experiment mussels Nos. 203 and 205 were placed side by side and subjected to the same conditions. No. 203 failed to open its siphons at all on this occasion, while No. 205, on the other hand, reacted to decreases in light intensity promptly and without any irregularities every minute for 50 minutes, at the end of which time the experiment was discontinued.

3. Comparative Measurements of Sensitivity

A sufficient number of experiments were performed on mussel No. 203 to make it possible to recognize a normal type of behavior for this individual. When it was responding normally, values of $\frac{A}{A+B}$ could be obtained which were comparable. This line of experimentation was not pursued very far, but some examples of the results obtained will be given. In one series of experiments (Nos. 42-46) conducted on different days, a number of tests were made in the course of each experiment. When the value of light B was allowed to remain constantly at 2 candle-meters, and the value of A varied till the inhibition point was reached, the values of $\frac{A}{A+B}$ were successively, for the different experiments, 0.14, 0.14, 0.10, 0.14, and 0.125, which show a fairly close degree of correspondence. In experiments Nos. 44-46, further tests were made with higher intensities of light. In these additional tests, the value of light A remained constantly at 22.2 candle-meters and the value of B was varied till the inhibition point was found. In these cases the value of $\frac{A}{A+B}$ at the inhibition point were successively 0.078, 0.08, 0.08, which series also shows close agreement. But the sensitivity in the latter case is seen to be very much greater than in the former. This increase may have been due to an increase in sensitivity accompanying the use of higher intensities of light, as was indicated on page 301, or to an increase in sensitivity due to previous stimulation (for the values for the higher intensities were obtained after those of the lower intensities) or to both causes. This line of experimentation, if pursued further, would permit the testing out the applicability of the Weber-Fechner law to the behavior of these animals.

The results obtained from these few experiments on the fresh-water mussel, while incomplete and fragmentary, nevertheless may indicate some lines of experimentation which might profitably be pursued further.

III. RESULTS WITH MARINE SPECIES

The experiments and results at Woods Hole may be grouped under two heads: (A) qualitative tests with different species, and (B) results with Pecten.

A. Qualitative Tests with Different Species

1. Classification Based on Sensitivity

Nagel ('94) has called the sense by which animals distinguish changes in light intensity "der photoskioptischen Sinn," or light-shadow sense, and speaks of animals as being "photoptic" or "skioptic," when they are sensitive respectively to increases or to decreases in light intensity. He gives a list of over 20 species of bivalves that he has tested and divides them into 6 classes, grading from purely skioptic through photoskioptic to purely photoptic. I find it more convenient to group the species tested at Woods Hole into 3 classes, viz.:

a. Sensitive both to increases and decreases.

1. Mya arenaria
2. Ensis directus
3. Venus mercenaria
4. Petricola dactylus
5. Spisula solidissima
6. Callocardia morrhuma

b. Sensitive to decrease only.

1. Pecten gibbus (var. borealis)
2. Ostrea virginiana
3. Modiola demissus (var. plicatula)
4. Modiola modiolus
5. Mytilus edulis
6. Liocardium mortoni
7. Anomia simplex (?)

c. Sensitive neither to increases nor decreases

1. Cumingia tellinoides
2. Yoldia limatula
3. Solenomya velum
4. Tellina tenera
5. Nucula proxima (?)

A question mark is placed after Nucula because the animal is so small and the siphons so short that slight movements may have been overlooked. So far as could be observed, however, there were no reactions. Since Nagel ('94) puts Tellina complanata and T. nitida rather high in the photoskioptic class, it may be that my failure to get responses from T. tenera was due to unfavorable physiological states of the animals experimented with. There is also some question about the position of Anomia simplex, for, although the animals tested reacted only to decreases, it is probable that the change from a depth of 8 to 10 meters—the level at which they were collected—to the surface caused a very great decrease in physiological tone. Such a loss in physiological tone was especially noticeable in Pecten and Arca collected from similar depths. In fact, Arca failed to give any consistent responses. I failed to find any species which reacted to increases only. Some of those in class *a* reacted more strongly to increases than to decreases, e.g., Venus, but all except those in class *c* reacted to decreases.

2. Relation of Sensitivity to Presence or Absence of Pigment

It is worthy of note that all the species named in classes *a* and *b* had more or less pigment deposited on the ends of the siphons, or along the edge of the mantle, as in Pecten and Ostrea; and these pigmented areas seemed in every case to be the parts sensitive to changes in the intensity of the light. In class *c* there was a total lack of pigment so far as I could discover by examining the living specimens. The correlation of the presence of pigment with the sensitive areas of these animals is in accordance with a general rule throughout the animal kingdom. That pigment is not necessary to such sensitivity has been well established by Beer ('01), R. Hesse ('02), and others. Nagel ('96) finds that Psammobia has no pigment on the siphons, yet it is very sensitive to increases in light intensity. On the other hand, I have found that Cummingia, which has relatively very long, non-pigmented, separate siphons, that move about actively, gave no sign of sensitivity to either increase or decrease in intensity in any of the two or three dozen animals that I tested. While the correlation between the presence of pigment and sensitivity is apparently perfect as far as my investigations go, this is not proof but that species may be found on our eastern coast for which this correlation fails to hold.

3. Differences in Reactions to Increase and Decrease of Light Intensity

Another point may be noted with respect to some members of class *a*, namely, that in certain cases, and especially in that of Mya, a different set of muscles is involved in the reaction to increase from that involved in the reaction to decrease of light intensity. Time after time specimens of Mya were tested and the reactions followed the same general rule, viz., for decreases in intensity, the movement was that of the muscles involved in closing the siphonal openings, while in responses to increases, the siphon tubes were withdrawn. In the latter case the withdrawal of the siphon tubes was sometimes accompanied by closure of the openings, especially if the reaction was a vigorous one, but as a general rule, when the movement of the siphon tube was not great, no closure of the openings resulted. Just why one set of muscles should be used for the reaction to increases and another set for reactions to decreases, is difficult to understand. Since Mya usually inhabits stretches of the shore which are exposed at low tide, it might be thought to be an adaptation to the conditions of its habitat for the siphons to be withdrawn as the tide goes out, accompanied as it would be with gradual increases in light intensity. But under the conditions of the experiments the reaction was to a sudden increase in the intensity of the light rather than to a slow or gradual increase. Another possibility is that the reaction is due to an increase in heat rather than to an increase in light. Experiments were not undertaken to determine this point. On the other hand, it is equally difficult to understand why decreases in intensity, which might accompany the approach of an enemy, were responded to by a mere closure of the siphonal openings, without a withdrawal of the siphon tube. A similar difference in reaction was noticed in Petricola and Spisula.

B. Results with Pecten

1. Sensitivity to Increase and Decrease

It will be noticed that Pecten is placed in class *b*, that is, among those which react to decrease only. Nagel ('94) classes Pecten and Arca among those which are "photoskioptic," but Dakin ('09) and Bauer ('12) say that Pecten does not react to increases in intensity. It is not strictly correct to say that Pecten does not react to increases, for at times sudden increases, especially when the light is very strong, will cause the animals to close their valves. But such reactions are very irregular and occur only after a latent period of several seconds (Nagel mentions the long latent period for reactions to increases). On the other hand, the reaction to decreases is nearly always immediate and regular. With respect to immediate and regular responses, therefore, it is proper to place Pecten gibbus (var. borealis) in class *b*. This property of Pecten seemed to offer an oppotunity to test the image-forming powers of its eyes by employing a moving object in such a way that it would be accompanied by an increase instead of a decrease in the intensity of the light.

a. Methods

For these experiments the following methods were employed. In a wooden box, about 45 x 30 x 30 centimeters, a hole about

4 centimeters in diameter was bored at one end near enough to the bottom to correspond to the height of the animals when they were placed in a dish containing sand and the dish was set in the box. On the outside of the box a circular disk of bristol board, blackened with India ink, was fastened at its center so that it could be rotated. Openings of various sizes were made in the disk near its periphery in such positions that they would coincide with the hole in the box when the disk was rotated. In a rough way, therefore, the amount of light falling on a given area of an animal could be controlled. For cutting off the light a separate black card was passed between the box and the light source (usually the window) close to the box but not touching it. Further precautions were taken to prevent vibrations, to which many species are very sensitive, by supporting the box and the dishes containing the animals on wads of cotton.

b. Results

Under the above conditions it was found that Pecten was very sensitive to decreases in light intensity, but reacted seldom to increases, and then only after a relatively long latent period. There was therefore little danger of confusing the two types of reactions. It was further observed that Pecten reacted more readily to decreases when the illumination was low, than when it was very bright. Low intensity corresponds more nearly to the conditions of their natural habitat than does high intensity, and it may be that their greater sensitivity at the lower intensities is an adaptation to their normal surroundings. Pecten differs in this respect from Anodonta, which lives in much shallower water.

Rawitz ('88) stated that a considerable number of eyes must be affected by a shadow in order to produce a response. Some attempts were therefore made to limit illumination to a small area of the mantle margin. By means of narrow slits or small round openings in the disk, it was possible to restrict the area subjected to bright illumination. In these cases direct sunlight was reflected from a mirror. In one instance only two eyes were thus illuminated, yet definite reactions followed the cutting off of the light. In several instances of this kind, where only a small area of the mantle edge of one side was illuminated, the response to a cutting off of the light was a very local muscular contraction involving little more than the illuminated area. These results are certainly quite different from those reported by Rawitz. Dakin ('10*a*) also states that a considerable number of eyes must be affected by a shadow in order to produce a reaction. Rawitz and Dakin, however, used small objects to produce local shadows instead of the local illumination method just described. The difference in method may account for the different results. The local responses obtained as a result of localised stimuli indicate their reflex character.

2. Reactions Toward a Moving Object

a. Experiments

For testing the image-forming powers of the eye in Pecten, the following methods were employed. A piece of bristol-board about 25 x 15 centimeters was blackened with India ink and pasted to a piece of heavy cardboard to give it rigidity. This was used as a background for the moving object, which was a white card 7.5 centimeters square. The animals were placed in the end of the box bearing the hole and the revolving disk, and the black bristol-board was stood up in the opposite end, that farthest from the window. To the white card a black thread was attached and passed vertically over a convenient gas jet—in lieu of a pulley—so that the card could be raised and lowered without the corresponding movement of the hand being visible to the animals.

First the animals were tested for increases and decreases in the usual way. Additional tests for increases were frequently made by flashing on to the animals a reflection of the light from the window by means of a mirror held in the hand. If the animals were found to be reacting normally, they were then tested with the moving card. Since the white card was receiving the light from the window, it will readily be understood that as the card moved upward from the bottom of the box, it would receive more and more light, and therefore would reflect more and more light on to the Pectens in the other end of the box. Each upward movement of the card would therefore be accompanied by an increase in the intensity of the light to which the animals were subjected. Any reaction they might give, therefore, could not be attributed to a decrease in the intensity of the light. Nevertheless, whenever the card was moved upward the animals gave immediate and vigorous responses by closing the valves or by contraction movements of the vela and tentacles. The reactions followed alike when the movement of the card was slow—i.e., about 5 centimeters per second—or when the card was suddenly jerked upward. Similar reactions were also obtained when the card was moved downward, or from side to side at a constant level. When a black card was substituted for a white one, reactions sometimes resulted, sometimes not. I cannot be certain whether there was enough contrast in the amount of light reflected from the background, compared to that from the card, to allow the animals to form an image of the moving black card, or whether some slight movement of the arm or body of the observer in connection with the pulling of the thread may not have been the source of the stimulus. That the eyes were concerned in the reception of the stimuli from the moving cards is indicated by the results of the following experiment. In all the previous experiments the animals were always placed with the open, or ventral, side toward the moving card. Now they were turned around 180 degrees so that their dorsal, or hinge, side was toward the card. In this position light from the card could not fall on the eyes and the animals gave no responses to the movement. Next they were turned back 90 degrees, so that either the anterior or posterior margins with their eyes were turned toward the card. In this position they gave fully as vigorous responses as when the ventral side was toward the card.

In one series of experiments the size of the white card was successively reduced to determine the lower limit of size to which the animals would respond. The smallest card, the movement of which produced a definite reaction, was 1.5 centimeters square and its distance from the animal about 35 centimeters. No attempt was made to determine a larger limit, nor any distance limits.

It is believed by the writer that these experiments with the upward moving white card on a black background, since any possibility of the movement being accompanied by a diminution of the intensity of the light was excluded, and since definite and vigorous responses were obtained, constitute a demonstration of the ability of the Pecten eye to form an image. Perhaps absolute proof could only be obtained by removing all the eyes and then subjecting the animals to the same tests. Such a method might also demonstrate whether the eyes alone, or other parts of the mantle-border as well, are sensitive to the changes in light intensity But the operation involved would undoubtedly be a great shock to the animals and their subsequent behavior might not be typical.

b. Discussion

The complex and highly organized structure of the eyes of Pecten has attracted the attention of histologists for many years. Those who have undertaken a careful study of the structure have usually found many difficulties to overcome, and unsatisfactory results were often obtained on account of imperfect technique. The most recent, and perhaps the most reliable, detailed study of the Pecten eye is that of Dakin ('10*b*), to which the reader is referred.

Many of those who have studied the histology of these eyes have assumed that the animals could "see" with them. The following quotation from Garner ('37) indicates the typical attitude among some of the earlier investigators: Garner says (p. 488): "In Pecten, Spondylus, and Ostrea we find small, brilliant, emerald-like ocelli, which, from their structure, having each a minute nerve, a pupil, a pigmentum, a striated body, and a lens, and from their situation at the edge of the mantle, where alone such organs could be useful, and also placed, as in Gasteropoda, with the tentacles, must be organs of vision." Similarly, on anatomical grounds, Grube ('40), Rawitz ('88), Schreiner ('97), R. Hesse ('00), and others conclude that these eyes are organs of vision. But very few of the histologists have performed crucial experiments to determine whether or not Pecten really could form an image. And some of them have indulged in various speculations as to some function other than vision.

As an example of such speculations I may quote Hickson ('80). He says (p. 454): "Little is known and little can be said concerning the function of the eyes of Pecten. The presence of such a well-formed tapetum makes it probable that they are capable of appreciating very diffused light. The close approximation of the lens to the retina makes it exceedingly improbable that any image is formed upon the latter. A few experiments have been made on the extent of their visual power, which make it very doubtful whether they are of much value to the animal in avoiding its enemies. The most reasonable theory of their function seems to be that, when on the ebbing tide, a probability arises that they will be left high and dry on the shore, they can appreciate the fact by the growing intensity of the light, and by the peculiar flapping motion of their valves the Pectens are so remarkable for, move away into deeper water."

Sharp ('87) criticises this idea of Hickson, calling attention to the well-formed eyes of the species found in the Mediterranean, where scarcely any tide exists. Earlier ('84), he had said (p. 458): " Now, I am inclined to believe, as has been suggested by Hensen ('65), that the pigmented epithelial cells that cover this organ and the stalk on which it is situated, and the only place where we find a marked deposit of pigment, is the seat of vision for these animals and that the so-called eye may be an organ of some other sense. . . . If the organ in question is so highly developed, we must necessarily conclude that vision, if that be the sense, is also highly developed, which does not appear to be the case. . . ." But in 1886 he offers the suggestion that they are phosphorescent organs, saying (p. 61): " It is not unreasonable to suppose that organs for emission of light would be constructed on the same principles as those for the admission of light." Dakin ('10*b*) tested out this phosphorescent theory and obtained only negative results.

Patten ('86) regarded the eyes of Pecten and Arca as organs for the absorption of energy from the sun, and termed them "heliophags." This idea has received no support from later investigators.

Attention should be called to the fact that Pectens, as well as other bivalves, fail to give consistent responses unless they are in good physiological condition. I found this to be true of all the different species experimented with, and in the case of Pecten I was able to get consistent responses only from those collected in very shallow water. Animals collected from a depth of 10 to 12 meters gave irregular responses and usually died at the end of two to four days in the laboratory aquaria.

As already stated, Rawitz ('88) claimed that no reactions were obtained from Pecten unless the shadow furnishing the stimulus affected several eyes, i.e., a considerable length of mantle edge. The shadow of a small object, such as a needle, brought no responses unless the shadow was made to fall on a series of eyes in quick succession, in which case the stimulation was the same as if a larger object had been used. From these results, and from the study of the structure of the eye, he concluded that the animals possessed a mosaic type of vision, saying (p. 548):

" Wird somit in jedem Auge nur ein kleiner Teil desjenigen Objectes abgebildet, welches sich gerade im Gesichtsfelde befindet, und sind zur deutlichen Wahrnehmung immer eine grossere Zahl von Augen notwendig (wie viel, weiss ich nicht; doch ist die Zahl der notwendigen Augen für diese Batrachtung nebensächlich), so heisst das· das Gesamtbild, welches eine Muschel wharnimmt, setzt sich aus einer bald grosseren, bald geringeren Zahl von Einzelbildchen zusammen, die linear aneinandergereiht sind. Wir haben es hier also mit einer Art musivischen Sehens, und zwar linearen musivischen Sehens zu thun." Rawitz made use of shadows in his experiments, thereby failing to make a distinction between reactions to decreases in the intensity of the light and reactions to the movement of an object. It would have been possible to have attributed the reactions he obtained to decreases in light intensity alone. As previously stated, I obtained responses with decreases when only two eyes were illuminated. Unfortunately, I did not attempt to determine the reaction of a single eye to the moving card in my experiments.

Nagel ('94) distinguishes between the sensitivity to changes of light intensity, which he called the "photoskioptic sense," and the sensitivity to the movement of an object. He says (p. 387): " Im Gegensatz dazu (photoskioptic animals) würden die ikonoptischen Tiere solche sein, bei welchen zu den photoskioptischen Wahrnehmungen der einzelnen sensiblen Elemente die Perception eines durch einen lichtbrechenden Apparat erzeugten Bildes hinzukommt." He regarded Pecten as "ikonoptic" and says ('96, p. 64): " Die verscheidenen Arten von Pecten, welche bekanntlich Augen in erheblichen Zahl am Mantelrande tragen, schliessen ihre Schalen schon, wenn man nur in the Nähe kommt, ohne dass ein Schatten auf sie gefallen wäre." While this latter statement is probably correct, these reactions might have been the result of very slight decreases in illumination due to the approach of the observer, or his passage between the animal and some of the windows of the room. I have obtained similar results under similar circumstances but regarded the observations as far less conclusive than those made in connection with the experiments with the moving card.

Greater conservatism is shown by some of the investigators, who have not jumped to unproved conclusions. Drew ('06, p. 50) says: " It is difficult to determine how well a scallop sees. . . . Quick motions outside of an aquarium made so the illumination is not materially affected and so the aquarium is not jarred, frequently seem to cause responses, but the results are so frequently negative that apparent responses may be accidental. Experiments to test the power of vision have not been devised." Differences in physiological states may account for the inconsistent results obtained by Drew.

Dakin ('10*b*) experimented on various kinds of Pecten and states (p. 102): " The eyes show no evidence of being phosphorescent organs, though I have observed and stimulated them at night and in the dark. A shadow thrown on to the eyes of an open Pecten causes a closure of the valves, and this reaction usually takes place very rapidly, though very often the perception of light stimuli does not appear to be any better than by Arca with very simple eyes or others with pigment spots." Dakin further states that no evidence of accommodation could be obtained experimentally. In regard to pigment spots it may be worth while to mention that the Pectens used in my experiments possessed, in addition to the eyes, pigment spots along the mantle edge similar to those found on the mantles of eyeless forms, and these pigment spots may be sensitive to changes in light intensity. However, the only method of testing the matter seems to be that of eliminating all the eyes and then subjecting the animals to changes of light intensity.

Only a few investigators have carried out careful experiments to determine the extent of the powers of vision in Pecten, and usually no adequate discrimination has been made between reactions to decreases in light intensity and the possible reactions to the movement of an object. Bauer and von Uexküll have performed the most critical experiments on Pecten. Bauer ('12, p. 134) says: " Es muss dagegen betont werden dass bei Pecten nicht die Harebsetzung der Lichtintensität den auslösenden Reiz darstellt, sondern dass das Tier die Bewegung des Schattens 'sieht'." In support of this conclusion he points out that when an opaque object is passed between the animal and the light source, there may be obtained an upper and a lower limit of rapidity with which the object can be moved and still produce a reaction. But in this experiment no distinction is made between the effect of the movement and that of a diminution of the light intensity. However, he relates that in preparing his camera for photographing the animals in the aquarium, the movement of his arm before the dark background of the black-cloth camera-cover was sufficient stimulus to cause a reaction. There was no shadow in this case, according to his statement. He also performed a series of experiments to determine the rôle of the eyes in the righting reflex ("Umkehrreflex") and concludes that they are vitally concerned in, and necessary for, the carrying out of this reflex.

Von Uexküll ('12) got the usual results with a shadow, i.e., closing of the shell. When, however, he placed a starfish—a natural enemy—on the glass side of an aquarium adjacent to the one in which the Pectens were confined, there was not the response that was to have been expected if the Pecten "saw" its enemy. The starfish was then made to move and the Pecten responded by extending its tentacles in the direction of the starfish. Dakin ('10*a*) showed that when fluid drawn from a chopped-up starfish was pipetted on to the tentacles of a Pecten it would close its shell or swim away. Von Uexküll argues, therefore, that a series of reactions is involved in the closure of the shell following a stimulation of the eyes by a moving object. The perception of the movement causes a positive reaction of the tentacles which extend toward the moving object. If the tentacles then receive a stimulation by some chemical substance, the shell is closed by the adductor muscles. In my experiments with the moving card there was no intermediate reaction of the tentacles, the reactions to the movement of the card being in nearly every case an immediate closure or partial closure of the valves. In the case of Von Uexkull's experiment, however, there seems to be no reason to doubt that the animals did react to the movement of the starfish, thus indicating the ability to form an image.

Thus, while some experimental evidence has been produced indicating an image-forming capacity for the eyes of Pecten, many of the experiments have been uncritical in that they have not been conducted so as to distinguish between reactions to decreases in light intensity and reactions to the movement of an object. The experiments and observations of Nagel, Bauer, and Von Uexküll, however, seem to be fairly conclusive. It is believed that the experiments described in the present paper were so conducted as to avoid any confusion of the two kinds of stimulation, and therefore, to establish that Pecten gibbus (var. borealis) responds to the movement of an object and consequently may be thought to form an image in its highly organized eyes.

IV. SUMMARY

A. Results on Anodonta: 1. Anodonta is sensitive to decreases in light intensity but not to increases. Responses were obtained to decreases so slight as not to be detected by the eye of the observer

2. Sensitivity is influenced by the physiological states of the animals in at least the following ways: (a) Animals kept in the dark a few hours are less sensitive than when kept in the light, even when the light is very weak (e.g , 4 or 5 candlemeters). (b) Animals are more sensitive after periods during which successive experimental stimulations were given than after periods of quiet. (c) Unnatural positions of repose for the animals seem to interfere with normal sensitivity. (d) The presence of foreign material in the mantle chamber may temporarily inhibit reactions to stimulation by decreases in light intensity. (e) The presence of eggs or embryos in the gills of gravid females appears to inhibit responses to decreases in the intensity of the light. (f) Inherent differences in sensitivity between individuals probably exists.

3. By letting A equal the amount of light (expressed in candlemeters) cut off to produce a decrease, and B equal the amount of uninterrupted light (expressed in candle-meters), the degree of sensitivity may be measured by the value of the fraction $\frac{A}{A + B}$ when A has the lowest value for which a response is given.

B. Results on marine species: 1. Three classes were distinguishable as follows: (a) Those sensitive to both increase and decrease in light intensity (e.g., Mya); (b) those sensitive to decrease only (e.g., Pecten); and (c) those sensitive neither to decreases nor to increases (e.g., Cumingia).

2. A perfect correlation was found for the animals studied, between sensitivity and the presence of pigment in the epithelium of the sensitive areas.

3. In the case of Mya arenaria, and some others, reaction to increases resulted in a withdrawal of the siphon tubes, while reaction to decreases resulted in a closure of siphonal openings, often unaccompanied by any withdrawal of the tube.

4. Experiments on Pecten, which is sensitive only to decreases (with exceptions noted in the text), demonstrated that: (a) vigorous reactions by closing the valves may be obtained when a white card is moved over a black background, even when the movement involves an increase in the intensity of the light falling on the animals. This is believed to show that the Pecten eye may form an image. (b) The smallest white card the movement of which produced a response at a distance of 35 centimeters was 15 millimeters square. (c) When bright illumination was employed reactions occurred on decrease in intensity even if only two eyes were affected. Response in this case was local.

V. LIST OF PAPERS CITED

Bauer, V. 1912. Zur Kenntnis der Lebensweise von *Pecten jacobaeus* L. in besonderen über die Funktion der Augen. *Zool. Jahrb., Abt. f. allg. Zool.*, Bd. 33, pp. 127-149.

Beer, T. 1901. Ueber primitive Sehorgane. *Wiener klin. Wochenschr.*, Jahrg. 14, pp. 255-261, 285-293, 314-324, 30 Textfig.

Dakin, W. J. 1909. Pecten. *Liverpool Marine Biol. Com., Mem.*, XVII, pp. viii+136, 9 pl., 4 text fig.

1910*a*. The Visceral Ganglion of Pecten, with some Notes on the Physiology of the Nervous System, etc. *Mitteil. Zool. Sta. Neapel*, Bd. 20, pp. 1-40, pl. 1-2, 1 text fig.

1910*b*. The Eye of Pecten. *Quart. Jour. Micr. Sci.*, Vol. 55, pp. 49-112, pl. 6-7, 2 text fig.

Drew, G. A. 1906. The Habits, Anatomy and Embryology of the Giant Scallop, *Pecten tenuicostatus* Mighels. *Univ. of Maine Studies*, No. 6, pp. 1-71, pl. 1-17.

Garner, R. 1837. On the Nervous System of Molluscous Animals. *Trans. Linn. Soc.*, Vol. 17, pp. 485-501, tab. 24-27.

Grube, A. E. 1840. Ueber Augen bei Muscheln. *Arch. f. Anat., Physiol., und wiss. Med.*, pp. 24-35.

Hensen, V. 1865. Ueber das Auge einiger Cephalopoden. *Zeitscnr. f. wiss. Zool.*, Bd. 15, pp. 155-242, Taf. 12-21.

Hess, C. 1910. Neue Untersuchungen uber den Lichtsinn bei wirbellosen Tieren. *Arch. ges. Physiol.*, Bd. 136, pp. 282-367, 6 Taf.

HESSE, R. Untersuchungen über die Organe der Lichtempfindung bei niederen
1900. Thieren. VI. Die Augen einiger Mollusken. *Zeitschr. f. wiss. Zool.*, Bd. 68, pp. 379-477, Taf. 25-32, 1 Textfig.
1902. Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. VIII. *Zeitschr. f. wiss. Zool.*, Bd. 72, pp. 565-656.

HICKSON, S. J. The Eye of Pecten. *Quart. Jour. Micr. Sci.*, Vol. 20, pp. 443-455,
1880. pl. 34-35.

NAGEL, W. A. Beobachtungen uber den Lichtsinn augenloser Muscheln. *Biol.*
1894. *Centralbl.*, Bd. 14, pp. 385-390.
1896. Der Lichtsinn augenloser Tiere. Jena, 8vo., 120 pp., 3 Textfig.

PATTEN, W. Eyes of Molluscs and Arthropods. *Mitteil. Zool. Sta. Neapel*, Bd.
1886. 6, pp. 544-756, pl. 28-32.

RAWITZ, B. Der Mantelrand der Acephalen. Erster Teil. Ostreacea. *Jena.*
1888. *Zeit. f. Naturw.*, Bd. 22, pp. 415-556, Taf. 13-18, 1 Text fig.

SCHREINER, K. E. Die Augen bei Pecten und Lima. *Bergens Museums Aarbog*
1897. for 1896, No. 1, pp. 1-51, 4 pl.

SHARP, B. On the Visual Organs in Lamellibranchiata. *Mitteil. Zool. Sta. Neapel,*
1884. Bd. 5, pp. 447-470, pl. 26.
1887. On the Eye of Pecten. *Proc. Acad, Nat. Sci.*, Philadelphia, 1886, pp. 61-62.

UEXKÜLL, J. v. Studien über den Tonus. VI. Die Pilgermuschel. *Zeitschr. f.*
1912. *Biol.*, Bd. 58, pp. 305-332, 6 Textfig.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

VOL. 1.

1. **COLE, L. J.** — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. **GULICK, A.** — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. **BOWDITCH, H.** — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. **COE, W. R.** — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. **BIGELOW, H. B.** — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
6. **MARK, E. L.** — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **BARBOUR, T.** — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
8. **BLACKMAN, M. W.** — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–343, 1 pl. Sept., 1905.
9. **CONGDON, E. D.** — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. **BLACKMAN, M. W.** — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pls. Feb., 1907.

VOL. 2.

11. **LINTON, E.** — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. **PARKER, G. H.** — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16) : 413–455. Apr., 1908.
13. **COLE, J. F.** — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
14. **MORGULIS, S.** — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
15. **MORGULIS, S.** — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
16. **RAND, H. W.** — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2) : 189–238, 2 pls. Sept., 1909.
17. **MARK, E. L.** — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate : 6 pp. Nov., 1909.
18. **SMALLWOOD, W. M.** — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1) : 137–145. June, 1910.
19. **SMALLWOOD, W. M., AND ROGERS, C. G.** — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
20. **CARPENTER, F. W.** — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6) : 147–162, 1 pl. Oct., 1910.
21. **ESTERLY, C. O.** — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7) : 217–226, 4 pls. July, 1911.
22. **COWDRY, E. V.** — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10** : 1–53, 4 pl. July, 1911.
23. **GROSS, A. O.** — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. **SMALLWOOD, W. M.** — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. **Mark, K. L.** — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. **Smallwood, W. M., and Clark, E. G.** — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23**(4) : 625–636. Dec., 1912.
27. **Chester, W. M.** — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48**(20) : 737–773, 4 pls. May, 1913.
28. **Kutchin, H. L.** — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49**(10) : 569–626, 8 pls. Oct., 1913.
29. **Hilton, W. A.** — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. **Crozier, W. J.** — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. **Parker, G. H.** — The Locomotion of Chiton. 2 p. April, 1914.
32. **Parker, G. H.** — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16**(3) : 443–446. April, 1914.
33. **Crozier, W. J.** — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **Crozier, W. J.** — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. **Crozier, W. J.** — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. **Arey, L. B.** — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. **Crozier, W. J.** — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. **Mavor, J. W.** — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. **Crozier, W. J.** — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. **Crozier, W. J.** — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.
41. **Crozier, W. J.** — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
42. **Hecht, S.** — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3) : 429–434. Apr., 1916.
43. **Crozier, W. J.** — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3) : 297–356. Apr., 1916.
44. **Crozier, W. J.** — Some Indicators from Animal Tissues. Jour Biol. Chem., **24** (4) : 443–445. Apr., 1916.
45. **Galloway, T. W., and Welch, P. S.** — Studies on a Phosphorescent Bermudian Annelid, Odontosyllis enopla Verrill. (Reprinted from Trans. Amer. Micros. Soc., **30** (1) : 13–39, 5 pls., Jan., 1911.) Reprint, 26 pp., 5 pls., June, 1916.
46. **Crozier, W. J.** — The Taste of Acids. Jour. Comp. Neurol., **26** (4) : 453–462. Aug., 1916.
47. **Wenrich, D. H.** — Notes on the Reactions of Bivalve Mollusks to Changes in Light Intensity: Image Formation in Pecten. Jour. Anim. Behavior, **6** (4) : 297–318. July–Aug., 1916.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

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34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
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38. Mayor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
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41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–278. Mar., 1916.
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45. Galloway, T. W., and Welch, P. S. — Studies on a Phosphorescent Bermudian Annelid, Odontosyllis enopla Verrill. (Reprinted from Trans. Amer. Micros. Soc., **30** (1) : 13–39, 5 pls., Jan., 1911.) Reprint, 26 pp., 5 pls., June, 1916.
46. Crozier, W. J. — The Taste of Acids. Jour. Comp. Neurol., **26** (4) : 453–462. Aug., 1916.
47. Wenrich, D. H. — Notes on the Reactions of Bivalve Mollusks to Changes in Light Intensity : Image Formation in Pecten. Jour. Anim. Behavior, **6** (4) : 297–318. July–Aug., 1916.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Nos. 48 and 49.

CELL PENETRATION BY ACIDS.

II. FURTHER OBSERVATIONS ON THE BLUE PIGMENT OF CHROMODORIS ZEBRA.

III. DATA ON SOME ADDITIONAL ACIDS.

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BY W. J. CROZIER.

Contributions from the Bermuda Biological Station for Research, No. 48.

(Received for publication, June 26, 1916.)

I. The blue substance occurring in the tissues of the large nudibranch *Chromodoris zebra* Heilprin has been employed as an intracellular indicator for acids, which on penetrating the tissues cause the material to become pink (Crozier, 1916). This paper records some further observations with reference to the action of the pigment as an indicator and to its participation in the natural color variations of *Chromodoris*.

It was at first considered not impossible that more than one substance might be concerned in the color change produced by acids. Smallwood (1910, p. 139) states that the five or six *white* conical elevations on the posterior border of the mantle of *Chromodoris* turn pink when treated with nitric acid; this statement, however, certainly involves an error, because the action of acids does not in any way affect the color of the creamy white contents of these glandular organs. Smallwood (unaware of the nature of the blue substance) was probably misled by the diffusion of pigment from neighboring portions of the integument, or by the pink hue assumed in pigment-bearing cells external to the body of these white glands.

It can be stated quite definitely that the blue substance is the only indicator involved, but its reactions are sufficiently complicated to require some attention. It would be of interest, in this connection, to examine the apparently similar blue pigments of other species of *Chromodoris*. According to Eliot's description (1913, p. 29, pl. II, fig. 9) the Japanese *Chromodoris marenzelleri* Bergh is colored in such a way as to suggest a skin pigment allied to that of *C. zebra*, as is also the case with some Pacific coast species (Cockerell, 1901).

II. The blue pigment also occurs dissolved in the blood of *Chromodoris*. The blood pigment in its reactions to alkalies and acids is identical with that obtained from the cells of the foot and general integument. The absorption spectra are likewise the same, a single band being present, which in neutral solutions centers at about 621 $\mu\mu$ (Crozier, 1914). Blood obtained by means of a syringe in such a way as to be kept from contact with air is of a deep blue, almost violet, color by reflected light, reddish purple by transmitted light. The reaction of blood obtained from the auricle was estimated from a number of analyses to be about $p_H = 7.4$ (20°C.), the procedure of Rowntree, Marriott, and Levy (1915) being used for this purpose.

Qualitative tests indicate the presence of manganese in the blood and in partially purified pigment extracts, but I am unable to say whether or not manganese forms a constituent of the pigment substance.

III. Undiluted blood and aqueous solutions of the pigment obtained from the integument were allowed to stand (a) in open vessels and (b) in tightly stoppered bottles; some of the latter contained air while others were completely filled with fluid. At 27°C. the pigment not in contact with air was rapidly decolorized; it was determined that the presence of pieces of tissue accelerated the decolorization. The reduction was slower at 20° than at 27°. In bottles holding air above the pigment solution the upper layer remained blue for some time, but ultimately was completely decolorized; in this case the blue color could be regenerated several times by shaking. The admission of fresh air caused these solutions again to become blue, provided they had not stood too long. The pigment solutions contained in open vessels were also decolorized after the formation of a bacterial film.

The addition of neutral H_2O_2 immediately produced a permanent intense blue coloration, or a violet tint showing blue fluorescence. This color was much more intense than could be produced by long shaking with air.

During the progress of decolorization there was never any indication of a pink stage. The color change with acids is therefore not a reduction.

Extracts of the pigment made with boiling water and subsequently preserved in closed vessels were not reduced, nor was there any evidence of reduction when the extraction was made with boiling water in a flask stoppered with cotton wool. Extracts prepared in the cold were not reduced in the presence of formaldehyde, $CHCl_3$, xylol, acetone, or pyridine. Numerous experiments were made by adding to solutions of the blue pigment, sterilized with $CHCl_3$, portions of the body of *Chromodoris* either whole or ground with sand. No reduction was obtained in this way even when the tests were carried out in tightly closed bottles containing no free air. Negative results were likewise obtained with the liver of fishes, which yields, according to Harris and Creighton (1915), a non-specific reductase rapidly affecting hemoglobin.

The reduction of this *Chromodoris* pigment seems, therefore, to be strictly comparable to that (oxyhemocyanin) found by Alsberg (1915) in the case of *Limulus*. The reduction is not due to enzymes, but to the action of putrefactive microorganisms. If to an aqueous extract of the pigment that has been sterilized by boiling there be added a similar solution that has become reduced, the inoculated flask is likewise slowly decolorized. When allowed to stand too long, the blue color cannot be restored even with H_2O_2, indicating that the blue substance has been destroyed.

Reduction of the blue material by direct chemical means is not easily effected. It is not decolorized by boiling, either alone or with acid, and it is only slowly reduced with zinc dust and acid. In the neutral or slightly alkaline condition the blue pigment is partially reduced to a greenish tint by Stoke's reagent, but it is not completely decolorized. Acid solutions are not reduced by Stoke's reagent. These tests with freshly prepared Stoke's reagent were checked spectroscopically.

Whether or not the blue pigment is concerned in respiration cannot as yet be decided, but its behavior certainly suggests that it may serve as an oxygen carrier. There is reason to believe that the metabolism of sluggish marine invertebrates, such as *Chromodoris*, is not of a rapid type (Vernon, 1895) and the difficulty with which the pigment seems to be reduced may thus be no real objection to its fulfilling a respiratory function. Some experiments made to test this question have not given definite results.

The method used in my previous work of handling the pieces of tissue has one advantage not mentioned in my first paper. In pressing the tissue gently between filter paper before submitting it to the action of acid, any blood pigment held in lacunar peripheral blood spaces is likely to be removed. Fortunately, it is possible to make a decisive test showing the absence of errors from this source. Some *Chromodoris* individuals show a pure white at the edge of the mantle, although their blood is blue, as is also the surface of the foot and other portions of the skin. Pieces of this white skin tissue do not turn pink with acid, even when first treated with H_2O_2 to oxidize any blood pigment present.

IV. Several of the factors involved in determining variations in the blue coloration of this animal may be distinguished: the presence or absence of the pigment in the integument, the details of its local distribution, the extent to which it is oxidized, the proportion of the pigment which is firmly bound by the cell protoplasm as compared with that present in solution, and the reaction of the fluid locally associated with the dissolved form of the pigment. The last two factors enumerated are the most important from the standpoint of the use of the blue pigment as an intracellular indicator. It should also be stated that the oxidized condition of the pigment is the only one which is turned pink by acids; if acid is added to a reduced solution, the solution remains colorless, becoming pink only when shaken with air or treated with H_2O_2.

These complicating factors are important in determining variations found in the acid penetration of mantle tissue from variously pigmented individuals; they are probably as much concerned at least as is the variable judgment of the observer, which I at first believed mainly responsible. As just stated, the cells of the blue integument appear to contain the pigment in two conditions: (a) a relatively free (dissolved) state, and (b) bound by the cell protoplasm in a firm combination which, as previously noted in the case of the dorsal skin, is affected only by strong acids. When pieces of the mantle are immersed in weak acid solutions the pink hue may disappear after a time, leaving the tissue blue; this is due to the fact that only the dissolved (acid-sensitive) portion of the pigment is diffusible. In working at low temperatures (15°) a serious source of confusion may arise here, since the outward diffusion of pigment is frequently more rapid than the inward progress of the acid. This difficulty is, however, practically absent in freshly collected animals. With the object of following the changes in pigmentation I have studied animals maintained in the laboratory for 3 to 4 months without food, and I am convinced that in these individuals the conditions as regards the blue pigment are materially altered from those normally obtaining. There is an increase in the relative amount of pigment not lost by diffusion into distilled water or acid, and the diffusible portion of the pigment is more *quickly* lost to a surrounding solution. The penetration time of all acids tested is much less than in freshly collected animals.

In discussing the results obtained by observing the comparative penetration speed of acids (Crozier, 1916), two circumstances were not dealt with which may be mentioned here. One is the possibility that the tissue does not remain inert when exposed to the action of acid; but is (in a sense) stimulated to oppose the entrance of the foreign material; the nature of this possible effect may be such as to involve the partial coagulation of the cell substance and an alteration in the relations of the internal pigment. Closely allied to this is the second possibility; namely, that coagulation may be induced by a variable fraction of the acid, while the remainder can affect the indicator less readily when the latter is bound (?) by coagulated protein. This source of objection is removed, I believe, by the following experiment:

When pieces of tissue which have been turned pink by acid are transferred to sea water, the acid diffuses away and sea water enters. This occurs within a few minutes, usually, depending on the acid and its concentration. The pink hue is then lost, the tissue becoming greenish blue because of the sea water alkalinity. If these pieces of tissue be then placed again in acid, they become pink almost instantly. This is true even with weak acids, as 0.05 N acetic.

V. The purple state of the indicator is the one most sensitive to the addition of acids, or rather, the solutions containing it are intermediate in actual acidity between those producing the blue and those producing the pink states. The small pigment globules within the cell may in some cases be of higher acidity than obtains in other cases, or in the rest of the cell contents; as Osterhout (1913) clearly pointed out, there are many semipermeable surfaces, probably of different qualities, comprised in the structure of a single cell. This means that, even if the protoplasm of blue and purple mantle tissues were penetrated by acids with equal ease, still in measuring penetration with this indicator the lowest

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(Received for publication, June 26, 1916.)

I. Several additional acids have been used in continuing a study of the penetration of living cells by acids, the object being to discover to what extent the visible penetration of diverse acids may be used to analyze the cell surface and the effect of various conditions upon it.[1] A description of the method according to which the indicator-containing tissue of the nudibranch *Chromodoris zebra* is utilized for this purpose has been given in a previous paper (Crozier, 1916). The present experiments having been made some months subsequent to those previously recorded, dilution curves were redetermined for acids (valeric, succinic) with which it was desired to compare the ones added to the list of eighteen first studied. In Table I the first and second series

TABLE I.

Penetration Time: A = First Series of Tests (Late Summer, 1915); B = Second Series (Spring, 1916). 27°C. (See Text.)

Dilution, normal.	Valeric.		Succinic.	
	A.	B.	A.	B.
liters	*min.*	*min.*	*min.*	*min.*
50	1.2	1.5	9.2	11.2
75	1.5	1.7		17.4
100	1.9	2.2	16.5	22.5
150	6.0	6.5		38
200	12.0	15.0	45	60
300	26.0	27.0		
400	55.0	62.0		

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[1] I am indebted to Professor E. L. Mark for assistance in obtaining material for this work.

of measurements for succinic and isovaleric acids are compared; the figures represent in each instance the mean of ten or more concordant experiments. The differences to be observed are in the main due, I believe, to seasonal fluctuations in the condition of the tissue experimented upon.[2] The nature of this seasonal fluctuation may, however, be fairly simple; it is not improbable that the lower temperature of these animals during the winter months is responsible for the differences found. All the measurements recorded were carried out at 27°C., which corresponds pretty closely to the temperature of *Chromodoris* in summer time, while in winter their natural temperature is at times as low as 15°. The animals employed for this work were living at 19–20°, and it is not to be expected that a brief elevation to 27° would have the same effect upon them as would a long residence at that temperature. Variations in the alkalinity of the sea water may also be significant.

The acids used were *n*-caproic, maleic, and fumaric. The solutions were made up in distilled water, and (as in the previous measurements) in some instances also in rain water; in both cases the results were the same. This apparent disregard of familiar osmotic and other effects has been quite deliberate, since it is proposed to use these data as a starting point from which to begin a study of the actions of individual salts.[3] Distilled water (or rain water) in the absence of acid produces, of course, an almost (but not quite) immediate increase in permeability, which may be easily detected by subsequently placing the tissue in acid (see Table II); but acids, even in very dilute solution, lead (Osterhout, 1914) to a decrease of permeability, even toward themselves, which removes much of the objection to this procedure.

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[2] It is perhaps necessary to state that the observed variations are not traceable to differences in the acid preparations. The temperature coefficients of acid diffusion will be considered in a subsequent paper.

[3] In this connection see Loeb and Cattell (1915) and Loeb (1915).

TABLE II.

The effect of preliminary treatment with distilled water upon the penetration time of acids. Typical data from one set of tests. Temperature = 24°C. The "control" gives the penetration time observed with similar tissue immersed directly in acid. There are added for comparison measurements with corresponding pieces of tissue killed by 1.0 per cent formaldehyde.

Animal.	In distilled H_2O.	Acid.	Time of penetration.	Control.	Dead tissue.
	min.		*min.*	*min.*	*min.*
A	3	0.05 N H_2SO_4.	2.5	5.0	2.0
	3	0.05 " "	2.3	5.1	1.8
B	2	0.1 N HCl.	1.5	1.8	0.9
	2	0.1 " "	1.3	2.1	1.2
	2	0.1 " "	1.5	2.1	1.1
				2.0	
C	1	0.1 N HCl.	2.1	2.0	1.0
	1	0.1 " "	2.0	2.5	1.0
	1	0.1 " "	3.2	1.9	
D	2	0.01 N valeric.	1.5	2.4	0.5
	2	0.01 " "	2.0	2.5	1.2
	2	0.01 " "	1.6	2.0	0.5

Table III contains a summary of the data on caproic, fumaric, and maleic acids, which are shown graphically in Fig. 1.

TABLE III.

The Penetration Time, Min., of Caproic, Maleic, and Fumaric Acids.

Acid.	Penetration time, 27 °C.										
Dilution	10.5	17.5	20	25	50	100	150	200	300	400	500
Caproic				2.3		4.7		9.3	17.0	25	6)
Maleic	3.6		4.4		8.1	19.4		28.0		55	
Fumaric		4.6	5.4		13.0	27.6	40.0	55.0	80.0		

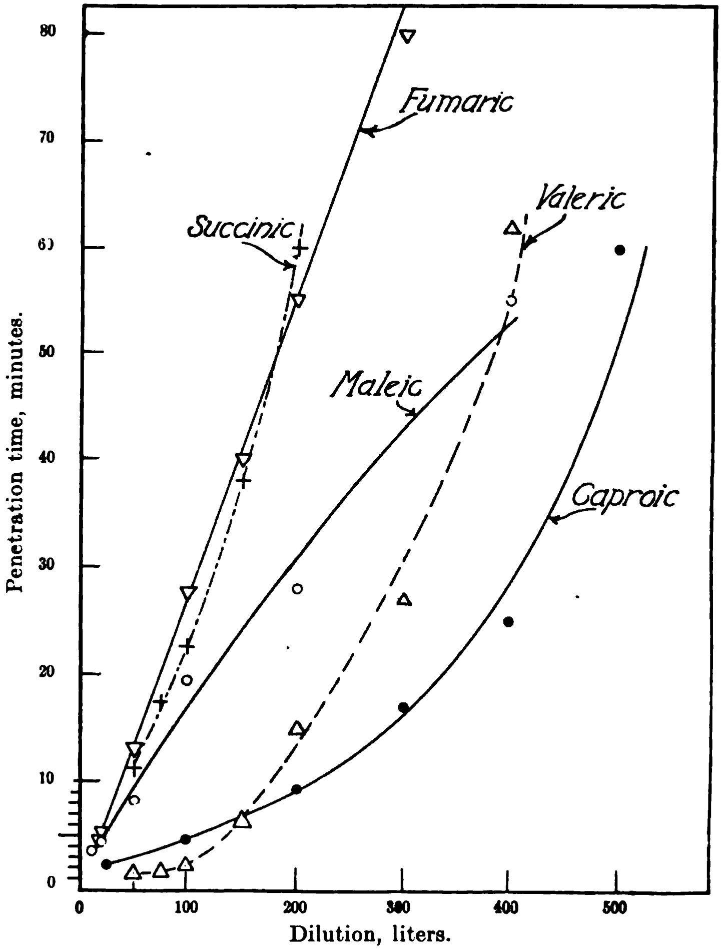

FIG. 1. Penetration of *Chromodoris* tissue. 27.0°.

II. The lipoid solubility of the monobasic fatty acids, aside from formic, follows the order shown in Table IV (quoted from Harvey, 1914, p. 468). This is precisely the order in which these acids, with the exception of caproic, had previously been found to affect the intracellular indicator of the *Chromodoris* tissues (Crozier, 1916). Caproic is now added to the list, and it will be noted that this acid also fits into the series (see Fig. 1).

TABLE IV.

Relative Lipoid Solubilities, Quoted from Harvey (1914).

Acid.	Caproic.	Valeric.	Butyric.	Propionic.	Acetic.
Partition coefficient. Xylol / water	3.5	0.6	0.1	0.02	0.00
Olive oil / water	7.25	1.78	0.44	0.14	0.05

The fact that in their first parts the curves for valeric and caproic acids are so near together, and even cross each other, does not interfere with this interpretation, since both are so far removed from the other weak fatty acids. The intracellular color change is an end result which involves several preliminary processes; one of these consists in the alteration of the cell surface to such an extent that the acid may enter; another consists in the diffusion of acid through the protoplasm. Provided the lipoid solubility of an acid be sufficiently great to speedily accomplish the former effect, it is not probable that an increase in lipoid solubility beyond this point would be markedly more efficient.

The view that this parallelism really indicates the method whereby these acids gain access to the interior of the integumentary cells of *Chromodoris* may be open to several qualifying objections, having reference to the variation of the partition coefficient with acid concentration, the nature of the lipoids possibly situated at the cell surface, and other similar points. There is, however, a correspondence between the penetration and solubility series, which is to a certain extent quantitative and cannot be ignored. Caproic and valeric acids are many times as soluble in olive oil or xylol as are the rest of the fatty acids, and they are similarly set apart from butyric, propionic, and acetic in the order of their cell penetrating ability. This is also true, in the same sense, for the concentrations of these acids necessary to reverse the phototropism of copepods (Loeb, 1911, p. 476). The combination of these acids with fatty substances situated at the cell surface enables the weak fatty acids to penetrate the cell in a sequence which has no reference to acid strength, either among themselves or in relation to other acids (compare Crozier, 1916).

III. Maleic and fumaric acids are of particular interest, because they may be closely compared with reference to the rôle of ionization in determining cell penetrating power. Harvey (1914) found that from 0.01 N solutions fumaric acid required 12 to 15 minutes, maleic acid 20 minutes, to penetrate the testis epithelium of *Stichopus ananus*. With this sequence my values (27.6 and 19.4 minutes, respectively) do not agree.

The near position of the curves for succinic, maleic, and fumaric acids, having respectively the ionization constants 0.00665, 1.17, and 0.093, is sufficient in itself to show that ionization is not the sole determinant of penetrating ability. Yet the order maleic > fumaric, succinic bears out the conclusion previously arrived at (Crozier, 1916) that, when closely related acids are considered, their sequence in affecting the intracellular indicator is controlled by acid strength, provided the ionization is neither too high nor too low.

SUMMARY.

IV. The penetrating ability of caproic, maleic, and fumaric acids has been tested for the indicator-containing tissue of the nudibranch *Chromodoris zebra*. The positions of these acids with relation to the eighteen previously studied are such as to support the conclusion derived from the latter; namely, that for any given acid at least two factors determine its relative position in the penetration series. One of these influences is ionization, the second concerns more particularly the union of the acid with one of the several constituents of the cell surface. In the case of the weak monobasic fatty acids, this constituent is of a fatty nature.

AGAR'S ISLAND, BERMUDA.

REFERENCES.

Crozier, W. J., 1916, Cell penetration by acids, *J. Biol. Chem.*, xxiv, 255–279.

Harvey, E. N., 1914, Cell permeability for acids, *Z. physik.-chem. Biol.*, i, 463–478.

Loeb, J., 1911, Die Tropismen, Winterstein's Handb. vergl. Physiol., Jena, iv, 451–519, 26 figs.

Loeb, J., 1915, On the rôle of electrolytes in the diffusion of acid into the egg of fundulus, *J. Biol. Chem.*, xxiii, 139–144.

Loeb, J., and Cattell, McK., 1915, The influence of electrolytes upon the diffusion of potassium out of the cell and into the cell, *J. Biol. Chem.*, xxiii, 41–66.

Osterhout, W. J. V., 1914, The effect of acid on permeability, *J. Biol. Chem.*, xix, 493–501.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. **Cole, L. J.** — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. **Gulick, A.** — The Fossil Land-Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. **Bowditch, H.** — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. **Coe, W. R.** — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow, H. B.** — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
6. **Mark, E. L.** — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **Barbour, T.** — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
8. **Blackman, M. W.** — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–343, 1 pl. Sept., 1905.
9. **Congdon, E. D.** — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. **Blackman, M. W.** — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. **Linton, E.** — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. **Parker, G. H.** — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16) : 413–455. Apr., 1908.
13. **Cole, J. F.** — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
14. **Morgulis, S.** — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
15. **Morgulis, S.** — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
16. **Rand, H. W.** — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2) : 189–238, 2 pls. Sept., 1909.
17. **Mark, E. L.** — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate : 6 pp. Nov., 1909.
18. **Smallwood, W. M.** — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1) : 137–145. June, 1910.
19. **Smallwood, W. M., and Rogers, C. G.** — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
20. **Carpenter, F. W.** — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6) : 147–162, 1 pl. Oct., 1910.
21. **Esterly, C. O.** — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7) : 217–226, 4 pls. July, 1911.
22. **Cowdry, E. V.** — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10** : 1–53, 4 pl. July, 1911.
23. **Gross, A. O.** — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. **Smallwood, W. M.** — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18): 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23** (4): 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20): 737–773, 4 pls. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10): 569–626, 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1): 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6): 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3): 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1): 8–20. Dec., 1914.
35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577): 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1): 37–44. July, 1915.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2): 196–202. Jan., 1915.
38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9): 183–211, 6 pls. Dec., 1915.
39. Crozier, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090): 735–736. Nov. 19, 1915.
40. Crozier, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1): 1–8. Feb., 1916.
41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3): 255–279. Mar., 1916.
42. Hecht, S. — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3): 429–434. Apr., 1916.
43. Crozier, W. J. — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3): 297–356. Apr., 1916.
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45. Galloway, T. W., and Welch, P. S. — Studies on a Phosphorescent Bermudian Annelid, Odontosyllis enopla Verrill. (Reprinted from Trans. Amer. Micros. Soc., **30** (1): 13–39, 5 pls., Jan., 1911.) Reprint, 26 pp., 5 pls., June, 1916.
46. Crozier, W. J. — The Taste of Acids. Jour. Comp. Neurol., **26** (4): 453–462. Aug., 1916.
47. Wenrich, D. H. — Notes on the Reactions of Bivalve Mollusks to Changes in Light Intensity: Image Formation in Pecten. Jour. Anim. Behavior, **6** (4): 297–318. July–Aug., 1916.
48. Crozier, W. J. — Cell Penetration by Acids. II. Further Observations on the Blue Pigment of Chromodoris zebra. Jour. Biol. Chem., **26** (1): 217–223. Sept., 1916.
49. Crozier, W. J. — Cell Penetration by Acids. III. Data on some Additional Acids. Jour. Biol. Chem., **26** (1): 225–230. Sept., 1916.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
2. Gulick, A. — The Fossil Land Shells of Bermuda. Proc. Acad. Nat. Sci. Phila., **56**(2) : 406–425, pl. 36. June, 1904.
3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July-Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
5. Bigelow, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15) : 557–592, 4 maps. Feb., 1905.
6. Mark, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54** : 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. Barbour, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7) : 107–134, 4 pls. Sept., 1905.
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9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
12. Parker, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts and Sci., **43**(16) : 413–455. Apr., 1908.
13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
14. Morgulis, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5) : 131–146. Nov., 1908.
15. Morgulis, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23) : 653–659, 1 pl. May, 1909.
16. Rand, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2) : 189–238, 2 pls. Sept., 1909.
17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate : 6 pp. Nov., 1909.
18. Smallwood, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda. Proc. Zoöl. Soc. London, **1910** (1) : 137–145. June, 1910.
19. Smallwood, W. M., and Rogers, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10) : 226–232. Apr., 1910.
20. Carpenter, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., **46**(6) : 147–162, 1 pl. Oct., 1910.
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22. Cowdry, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10** : 1–53, 4 pl. July, 1911.
23. Gross, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1) : 49–71, pls. 3–11. Jan., 1912.
24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

VOL. 3.

25. **MARK, K. L.** — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18): 669–678. April, 1913.
26. **SMALLWOOD, W. M., AND CLARK, E. G.** — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23**(4): 625–636. Dec., 1912.
27. **CHESTER, W. M.** — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48**(20): 737–773, 4 pls. May, 1913.
28. **KUTCHIN, H. L.** — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49**(10): 569–626, 8 pls. Oct., 1913.
29. **HILTON, W. A.** — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1): 113–130. Dec., 1913.
30. **CROZIER, W. J.** — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6): 491–492. Febr., 1914.
31. **PARKER, G. H.** — The Locomotion of Chiton. 2 p. April, 1914.
32. **PARKER, G. H.** — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16**(3): 443–445. April, 1914.
33. **CROZIER, W. J.** — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol.
34. **CROZIER, W. J.** — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1): 8–20. Dec., 1914.
35. **CROZIER, W. J.** — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577): 28–36. Jan., 1915.
36. **AREY, L. B.** — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1): 37–44. July, 1915.
37. **CROZIER, W. J.** — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2): 196–202. Jan., 1915.
38. **MAVOR, J. W.** — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9): 183–211, 6 pls. Dec., 1915.
39. **CROZIER, W. J.** — On Cell Penetration by Acids. Science, N. S., **42** (1090): 735–736. Nov. 19, 1915.
40. **CROZIER, W. J.** — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1): 1–8. Feb., 1916.
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48. **CROZIER, W. J.** — Cell Penetration by Acids. II. Further Observations on the Blue Pigment of Chromodoris zebra. Jour. Biol. Chem., **26** (1): 217–223. Sept., 1916.
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FOR RESEARCH.

No. 50.

ON A BARNACLE, CONCHODERMA VIRGATUM, ATTACHED TO A FISH, DIODON HYSTRIX.

By W. J. Crozier.

From The American Naturalist, Vol. L, No. 598.

CAMBRIDGE, MASS., U.S.A.
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ON A BARNACLE, CONCHODERMA VIRGATUM, ATTACHED TO A FISH, DIODON HYSTRIX[1]

A SPECIMEN of the "sea porcupine," *Diodon hystrix* Linn., seen swimming near the surface and secured with a dipnet, was found to have two living lepad barnacles attached to one of its erectile spines[2] upon the ventral surface two centimeters to the right anteriad of the anus. The *Diodon* was a small individual, 16 cm. long. It was kept under observation in the laboratory for several weeks.

According to a determination for which I am indebted to Mr. H. G. Coar, the barnacles belong to the species *Conchoderma virgatum* (Spengler), although varying "a trifle from Gruvel's type description, but not sufficiently to correspond to *Conchoderma hunteri* R. Owen, 1830, which the specimen approached slightly, nor to Leach's (1818) variety *chelonophilus* of *C. virgatum.*" This species has not previously been recorded from the Bermuda area, though it is known over the Atlantic generally and (to judge from statements of fishermen) occurs here upon young turtles. *C. virgatum* has been found on *Mola,* ships' bottoms, and various other objects (Pilsbry, 1907, p. 99), but the present record is somewhat unusual.

Different semiparasitic lepads have quite various hosts, such as medusæ, antipatharians, the spines of echinoids, molluscs, crustaceans, sharks, teleosts, turtles, the tail feathers of sea birds, whales, and so forth (Pilsbry, 1907; 1910). Those occurring on fishes seem, naturally, to affix themselves to some hard part, for example, the head, as in the case of *Tylosurus* (Sumner, Osburn, and Cole, 1913, p. 647). Jordan (1905, p. 341, fig. 226) figures a flying fish with conchodermas attached to a *Penella* growing on the fish, a condition of double parasitism which has been described for Xiphias. In the present instance, the larger of the two conchoderma individuals (20 mm. long) was found to have its peduncle completely surrounding the spine to which it had become fixed. The second individual was much smaller (4 mm. long) and attached to the peduncle of the first. Both specimens were so oriented that the opening between the valves was directed toward the head of the fish. The skin of the fish about the base of the spine was inflamed, and the muscles which normally control its elevation for defensive purposes had apparently degenerated. When it was attempted to preserve the *Diodon*, the spine bearing the conchodermas became detached in the course of the animal's self-inflation. It is probable, therefore, that the spine would soon have been shed under natural circumstances.

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[1] Contributions from the Bermuda Biological Station for Research, No. 50.

[2] The figure of *Diodon hystrix,* which is used in current ichthyological handbooks, represents the animal in a semipuffed-up condition and with the frontal spines erected. Alive, the fish has a quite different aspect, all the spines being flattened down to the skin unless the creature is much disturbed. When preserved in formalin it assumes the appearance depicted in the handbooks.

Several features of the behavior of these conchodermas are of interest in comparison with those of other barnacles. Some years ago it was reported by Pouchet et Joubert (1876) that cirripedia attached to rocks reacted to shading, while those attached to floating objects did not; their inference being that to the stationary barnacles a shadow signified danger, whereas, to those borne about at the surface of the water, a fluctuating illumination was the normal state of affairs. This observation has been regarded as an instance of adaptation comparable with that of Hargitt (1909) on the gradual loss of reaction to shading when serpulids are maintained in the laboratory.

The specimens of *Conchoderma* attached to *Diodon* did not react to shadows under any of a number of experimental conditions. They seem, therefore, to be in agreement with the observation of Pouchet et Joubert. But tests upon lepads found upon floating timbers and upon *Ascophyllum* showed that *Lepas anserifera* and *L. pectinata* do respond to shading by retracting the legs and approximating the valves. From a number of tests it appeared that neither the legs nor valves are sensitive to shading, but that the shadow must affect some part of the body within the shell suggesting that the persisting nauplius eye is the organ involved. The extent of the response varies with the degree to which the appendages have been extruded: when just being extruded, they react by complete retraction; when fully extruded, by a partial retraction; after being fully extruded for one or two minutes, they react to shading quite promptly and completely. After completion of a response there must usually elapse from two to four minutes before another reaction can be secured.

It seems to me, then, that the supposed adaptation of floating barnacles is not of the nature which has been supposed. Whether the non-reaction of *Conchoderma* to shading is properly to be considered a direct adaptation is therefore questionable. The host of these particular specimens is not a surface fish, and the absence of sensitivity to shading may be due to their deep habitat. Direct sunlight inhibited the rhythmic movements of the conchodermas, and they were much more active at night than in diffuse laboratory light.

The statement is occasionally met with that in barnacles attached to a free-swimming animal the feathery feet are merely thrust out, not waved about as in the rock barnacles, which must create food- and respiratory-currents for themselves. Now, it was observed that when the *Diodon* bearing the conchodermas was actively swimming, the legs of the lepads remained extended for as much as four to five minutes; whereas, when the fish remained stationary, they were alternately extended and retracted about seven times every minute (at 18° C.), the extension in the latter case being not so great as when their host was moving. *Lepas anserifera* and *L. pectinata* were then tested as to their behavior in currents, with this result: when the wood to which they were attached was stationary, the rhythmic contraction of the appendages was continuous, but if a gentle stream of water from a supply jet was allowed to flow past them impinging on the anterior (concave) edges of the legs, they remained extended for as long as ten minutes, and were spread farther apart than in the absence of the current. This was not due to any merely mechanical effect of the water stream, as the feet could at any time be caused to contract at a touch. A water stream, striking the posterior (convex) edges of the legs, led to contraction and subsequent limited extrusion of these appendages. A more correct interpretation of the phenomenon described in floating barnacles seems to be, therefore, that when the concave side of the appendages is stimulated by a water current, the animal responds by pushing out its legs further than is usual in the absence of currents, while their rhythmic contraction is inhibited. It should be noted that the two specimens of *Conchoderma* observed were so oriented on the *Diodon* as to receive the full benefit of currents derived from its forward swimming; and further, that this fish is not a vigorous swimmer, so that the currents in question are by no means rapid, but rather such as could be efficiently strained by the barnacles.

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REFERENCES

Hargitt, C. W. 1909. Further Observations on the Behavior of Tubicolous Annelids. *Jour. Exp. Zool.*, Vol. 7, pp. 157–187.

Jordan, D. S. 1905. A Guide to the Study of Fishes. Vol. I, 4°, xxvi + 624 pp., 393 figs. New York.

Pilsbry, H. A. 1907. The Barnacles (*Cirripedia*) contained in the Collections of the U. S. National Museum. Bull. U. S. Nat. Mus., 60, x + 122 pp., 11 pl. Wash.

Pilsbry, H. A. 1910. *Stomatolepas*, a Barnacle Commensal in the Throat of the Loggerhead Turtle. AMER. NAT., Vol. 44, pp. 304–306, 1 fig.

Pouchet, et Joubert. 1876. La vision chez les Cirrhipèdes. *C. r. et Mém. Soc. Biol.*, Sér. 6, t. 2, pp. 245–247.

Sumner, F. B., Osburn, R. C., and Cole, L. J. 1913. A Biological Survey of the Waters of Woods Hole and Vicinity, Section III. A Catalogue of the Marine Fauna. Bull. U. S. Bur. Fish., Vol. 31 (1911), Pt. 2, pp. 545–794.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

Vol. 1.

1. Cole, L. J. — Pycnogonida collected at Bermuda in the Summer of 1903. Proc. Bost. Soc. Nat. Hist., **31**(8) : 315–328, pls. 20–22. July, 1904.
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3. Bowditch, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452) : 555–563. July–Aug. [Sept.], 1904.
4. Coe, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10) : 531–570, pls. 23–25. Nov., 1904.
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8. Blackman, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13) : 329–343, 1 pl. Sept., 1905.
9. Congdon, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18) : 461–485. Jan., 1907.
10. Blackman, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19) : 487–518, 2 pls. Feb., 1907.

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13. Cole, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2) : 49–56, 1 chart. June [Aug.], 1908.
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17. Mark, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate : 6 pp. Nov., 1909.
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28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569–626, 8 pls. Oct., 1913.

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35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.

36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.

37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.

38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.

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40. Crozier, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH.

VOL. 1.

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VOL. 2.

11. Linton, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560) : 85–126, pls. 1–15. Oct., 1907.
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24. Smallwood, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16) : 607–630. March, 1912.

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Vol. 3.

25. Mark, E. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin: a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773, 4 pls. May, 1913.
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32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
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36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION
FOR RESEARCH.

No. 51.

ON THE IMMUNITY COLORATION OF SOME NUDIBRANCHS.

By W. J. Crozier.

From The Proceedings of the National Academy of Sciences,
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ON THE IMMUNITY COLORATION OF SOME NUDIBRANCHS

By W. J. Crozier

BERMUDA BIOLOGICAL STATION FOR RESEARCH, AGAR'S ISLAND, BERMUDA*

Read before the Academy, November 14, 1916. Received, November 8, 1916

Certain large nudibranchs, notably species of *Chromodoris*, exhibit a combination of brilliancy of pigment pattern and boldness of habits sharply distinguishing them from related nudibranchs which appear to be efficiently protected by their concealing coloration and behavior. It has long been suspected that the strikingly colored chromodorids achieve an immunity from predatory enemies by virtue of some distasteful quality, according to the classical conception of 'warning coloration.' The experimental evidence for this belief, as presented by Herdman and by Crossland, has remained, however, very fragmentary and is indeed far from carrying conviction.

For several years I have been interested in the pigmentation of the large Bermudian *Chromodoris zebra* Heilprin, mainly because its blue integumentary pigment is available as an intracellular indicator of acidity. On the basis of field observations, continuous throughout the year, I was led to undertake a series of experiments intended to throw light upon the possible biological meaning of this animal's coloration. The outcome seems reasonably conclusive, and it leads to the rejection of the idea that a warning significance attaches to the pigmentation of *Chromodoris*. By inference, one is inclined to suspect that more complete study will reveal analogous conditions with reference to other brightly colored nudibranchs.

The pigment pattern of *C. zebra* is, in brief, an irregular streaking of yellow or orange upon a field of blue. The foot, the ventral surface of the mantle, and various less conspicuous parts are of a pure blue. The animals occur at various stations, ranging in depth from about 10 fathoms to the surface of the water. They are most abundant, though, in quite shallow water, except during a portion of the summer and immediately after severe storms at other seasons. Their coloration is always conspicuous, not only because of its brilliancy but also by reason of the freedom with which they expose themselves in bright sunlight upon bare open bottoms or (a favorite situation) on eel grass in tidal 'creeks.' The animals are positively phototropic. Their conspicuousness is entirely independent of their being viewed form above the water surface.

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*Contributions from the Bermuda Biological Station for Research, No. 51.

Less than two per cent of the specimens collected are found to show evidence of mutilation. As these nudibranchs regenerate very slowly (Child, and my own observations), this evidence is significant.

Experiments in feeding entire, undamaged specimens of this nudibranch to other animals, both in natural surroundings and under favorable conditions in aquaria, have shown that to all the animals usually associated with *Chromodoris* its flesh is repugnant. This is true of fishes as well as invertebrates, including anemones, crustacea, various worms, and starfish. Some fishes which feed by night (squirrel fishes), and others which hunt their prey by sight (snappers), will make several attempts to bite a *Chromodoris* when it is first dropped into their aquarium tank, but thereafter will not approach it; after the nudibranch has sunk to the bottom and begun its normal creeping, it is never seriously molested.

The unpleasant quality is associated with the skin, since the internal organs are greedily devoured. The blue pigment is not the unpleasant substance, for the intensely blue rhinophores are eaten readily, while the blood, which I have elsewhere shown to contain the blue skin pigment (presumably as a respiratory chromogen of some sort), evokes positive food-taking responses from various fishes, crabs, and anemones. The yellow pigment is not the responsible agent, as it is absent from the edge of the mantle, a region which comparative tests indicate to be the most 'unpleasant' part of the animal's body.

Small pieces of the skin of *Chromodoris* will be snapped at several times by a fish (over twenty species have been tested), while an intact nudibranch will usually be merely approached, 'nosed,' and perhaps bitten once, if the fish swims up suddenly, before it is left alone. The explanation of this behavior is found in the fact that when the intact nudibranch is locally disturbed by being handled severely, bitten, cut, stung by nematocysts, or stimulated with induction shocks, it excretes from the irritated area, into the sea water, a bluish-white material which causes immediate negative reactions in fishes and in all classes of marine invertebrates which I have tested. Isolated portions of the *Chromodoris* skin, however, give the reaction with difficulty, if at all, and then only to a slight extent.

It is significant that the secretion comes mostly from the ventral surface of the mantle. When this nudibranch is disturbed, its first reaction is a general contraction, in which the mantle edge is extended and made prominent by internal fluid pressure; the edge of the mantle is invariably the region which is bitten at by fishes, and it is only the part which is damaged in the small number of mutilated specimens found in the field. There exists here, I believe, an interesting case of adaptive correlation.

As excreted, the repelling material contains globules of the blue skin pigment, which seems to be accidentally included in the discharged substance; but it is mainly a coagulated white substance holding oily globules. It is this substance which is the essential repellant.

There is still another phase of the matter, which is the most significant of all. A striking characteristic of these nudibranchs is the curiously penetrating, disagreeable odor which attends them even when they are undisturbed. To this is to be assigned responsibility, I believe, for the facts, (1) that cloth bags containing *Chromodoris* are avoided by fishes; (2) that blinded fishes avoid them; and (3) that animals never normally in contact with *Chromodoris*, such as the dogfish, remora, and the hawksbill turtle, will approach this nudibranch closely, then quickly retreat without touching it. The olfactory locus of these reactions seems probable, but it does not necessarily involve the idea of 'warning odor.'

In the present instance we have, in my opinion, positive proof for the validity of the theory of immunity coloration developed by Reighard. According to this view, startling colors are in many (if not in most) cases "conceived to have arisen through internal forces under immunity of the organism from the action of selection on its characters." The immunity of *Chromodoris zebra* is determined by the repugnatorial character of skin secretions under the control of its nervous system, and is further made effective by a repelling odor, which we have reason to believe stimulates the olfactory organs of fishes at least. Its success in maintaining itself as a plentiful inhabitant of the shoal waters of a 'coral' reef region is conditioned by this immunity and by the unpalatable nature of the jelly surrounding its egg strings; the slow rate of development of its eggs is counterbalanced by the fact that the animals reproduce throughout the year.

The coloration of *C. zebra* is a metabolic accident, at least in relation to its protection, for a single experience with a normally colored specimen is sufficient to cause snappers, turbots, and groupers to have nothing to do with subsequent individuals offered to them, even though these individuals are stained red or blue.

Conceivably, its color may at times serve to warn predatory foes, but the evidence herein adduced makes it clear that the pigmentation of *Chromodoris* plays no necessary rôle of this kind. We are consequently at liberty to infer that the conspicuous coloration did not develop, as the result of selection, according to the scheme proposed by the theory of warning coloration.

An illustrated account of this work will be submitted for publication later.

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10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

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3. **Bowditch**, H. — A List of Bermudian Birds seen during July and August, 1903, Amer. Nat., **38**(451, 452): 555–563. July–Aug. [Sept.], 1904.
4. **Coe**, W. R. — The Anatomy and Development of the Terrestrial Nemertean (Geonemertes agricola) of Bermuda. Proc. Bost. Soc. Nat. Hist., **31**(10): 531–570, pls. 23–25. Nov., 1904.
5. **Bigelow**, H. B. — The Shoal-Water Deposits of the Bermuda Banks. Proc. Amer. Acad. Arts and Sci., **40**(15): 557–592, 4 maps. Feb., 1905.
6. **Mark**, E. L. — The Bermuda Islands and the Bermuda Biological Station for Research. Proc. Amer. Assoc. Adv. Sci., **54**: 471–501, 16 pls. Dec., 1905. *Also separate*, 32 pp., 16 pls. Feb., 1905.
7. **Barbour**, T. — Notes on Bermudian Fishes. Bull. Mus. Comp. Zoöl. Harvard Coll., **46**(7): 107–134, 4 pls. Sept., 1905.
8. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. IV. On the Karyosphere and Nucleolus in the Spermatocytes of Scolopendra subspinipes. Proc. Amer. Acad. Arts and Sci., **41**(13): 329–343, 1 pl. Sept., 1905.
9. **Congdon**, E. D. — The Hydroids of Bermuda. Proc. Amer. Acad. Arts and Sci., **42**(18): 461–485. Jan., 1907.
10. **Blackman**, M. W. — The Spermatogenesis of the Myriapods. V. On the Spermatogenesis of Lithobius. Proc. Amer. Acad. Arts and Sci., **42**(19): 487–518, 2 pls. Feb., 1907.

Vol. 2.

11. **Linton**, E. — Notes on Parasites of Bermuda Fishes. Proc. U. S. Nat. Mus., **33**(1560): 85–126, pls. 1–15. Oct., 1907.
12. **Parker**, G. H. — The Sensory Reactions of Amphioxus. Proc. Amer. Acad. Arts. and Sci., **43**(16): 413–455. Apr., 1908.
13. **Cole**, J. F. — Magnetic Declination and Latitude Observations in the Bermudas. Terrestrial Magnetism and Atmospheric Electricity, **13** (2): 49–56, 1 chart. June [Aug.], 1908.
14. **Morgulis**, S. — The Effect of Alkaloids on the Early Development of Toxopneustes variegatus. Proc. Amer. Acad. Arts and Sci., **44**(5): 131–146. Nov., 1908.
15. **Morgulis**, S. — Regeneration in the Brittle-Star Ophiocoma pumila, with Reference to the Influence of the Nervous System. Proc. Amer. Acad. Arts and Sci., **44**(23): 653–659, 1 pl. May, 1909.
16. **Rand**, H. W. — Wound Reparation and Polarity in Tentacles of Actinians. Jour. of Exp. Zoöl., **7**(2): 189–238, 2 pls. Sept., 1909.
17. **Mark**, E. L. — The New Bermuda Biological Station for Research. Proc. Seventh Internat. Zoöl. Congress, Boston Meeting.
    Separate: 6 pp. Nov., 1909.
18. **Smallwood**, W. M. — Notes on the Hydroids and Nudibranchs of Bermuda Proc. Zoöl. Soc. London, **1910** (1): 137–145. June, 1910.
19. **Smallwood**, W. M., **and Rogers**, C. G. — Studies on Nerve Cells. III. Some metabolic Bodies in the Cytoplasm of Nerve Cells of Gasteropods, a Cephalopod, and an Annelid. Anat. Anz., **36** (8–10): 226–232. Apr., 1910.
20. **Carpenter**, F. W. — Feeding Reactions of the Rose Coral (Isophyllia). Proc Amer. Acad. Arts and Sci., **46**(6): 147–162, 1 pl. Oct., 1910.
21. **Esterly**, C. O. — Calanoid Copepoda from the Bermuda Islands. Proc. Amer. Acad. Arts and Sci., **47**(7): 217–226, 4 pls. July, 1911.
22. **Cowdry**, E. V. — The Colour Changes of Octopus vulgaris Lmk. Univ. Toronto Studies. Biol. Ser., No. **10**: 1–53, 4 pl. July, 1911.
23. **Gross**, A. O. — Observations on the Yellow-billed Tropic-bird (Phaëthon Americanus Grant) at the Bermuda Islands. Auk, **29**(1): 49–71, pls. 3–11. Jan., 1912.
24. **Smallwood**, W. M. — Polycerella zoobotryon (Smallwood). Proc. Amer. Acad. Arts and Sci., **47**(16): 607–630. March, 1912.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. (*Continued.*)

Vol. 3.

25. Mark, K. L. — Preliminary Study of the Salinity of Sea-water in the Bermudas. Proc. Amer. Acad. Arts and Sci., **48** (18) : 669–678. April, 1913.
26. Smallwood, W. M., and Clark, E. G. — Chromodoris zebra Heilprin : a Distinct Species. Jour. of Morph., **23** (4) : 625–636. Dec., 1912.
27. Chester, W. M. — The Structure of the Gorgonian Coral Pseudoplexaura crassa Wright and Studer. Proc. Amer. Acad. Arts and Sci., **48** (20) : 737–773, 4 pls. May, 1913.
28. Kutchin, H. L. — Studies on the Peripheral Nervous System of Amphioxus. Proc. Amer. Acad. Arts and Sci., **49** (10) : 569–626, 8 pls. Oct., 1913.
29. Hilton, W. A. — The Central Nervous System of Tunica nigra. Zool. Jahrb., Abt. f. Anat., **37** (1) : 113–130. Dec., 1913.
30. Crozier, W. J. — Note on the Pigment of a Bermuda Nudibranch, Chromodoris zebra Heilprin. Jour. of Physiol., **47** (6) : 491–492. Febr., 1914.
31. Parker, G. H. — The Locomotion of Chiton. 2 p. April, 1914.
32. Parker, G. H. — On the Strength and the Volume of the Water Currents produced by Sponges. Jour. of Exp. Zoöl. **16** (3) : 443–445. April, 1914.
33. Crozier, W. J. — The Sensory Reactions of Holothuria surinamensis Ludwig. Zool. Jahrb., Abt. f. Physiol., **35** (3) : 233–297. Aug., 1915.
34. Crozier, W. J. — The Orientation of a Holothurian by Light. Amer. Jour. of Physiol., **36** (1) : 8–20. Dec., 1914.
35. Crozier, W. J. — On the Number of Rays in Asterias tenuispina Lamk. at Bermuda. Amer. Nat., **49** (577) : 28–36. Jan., 1915.
36. Arey, L. B. — The Orientation of Amphioxus during Locomotion. Jour. of Exp. Zoöl., **19** (1) : 37–44. July, 1915.
37. Crozier, W. J. — A Note on the Physiology of the Cuvierian Organs of Holothuria captiva Ludw. Amer. Jour. of Physiol., **36** (2) : 196–202. Jan., 1915.
38. Mavor, J. W. — On the Development of the Coral Agaricia fragilis Dana. Proc. Amer. Acad. Arts and Sci., **51** (9) : 183–211, 6 pls. Dec., 1915.
39. Crozier, W. J. — On Cell Penetration by Acids. Science, N. S., **42** (1090) : 735–736. Nov. 19, 1915.
40. Crozier, W. J. — Regarding the Existence of the 'Common Chemical Sense' in Vertebrates. Jour. Comp. Neurol., **26** (1) : 1–8. Feb., 1916.
41. Crozier, W. J. — Cell Penetration by Acids. Jour. Biol. Chem., **24** (3) : 255–279. Mar., 1916.
42. Hecht, S. — The Water Current produced by Ascidia atra Lesueur. Jour. of Exp. Zoöl., **20** (3) : 429–434. Apr., 1916.
43. Crozier, W. J. — The Rhythmic Pulsation of the Cloaca of Holothurians. Jour. of Exp. Zoöl., **20** (3) : 297–356. Apr., 1916.
44. Crozier, W. J. — Some Indicators from Animal Tissues. Jour. Biol. Chem., **24** (4) : 443–445. Apr., 1916.
45. Galloway, T. W., and Welch, P. S. — Studies on a Phosphorescent Bermudian Annelid, Odontosyllis enopla Verrill. (Reprinted from Trans. Amer. Micros. Soc., **30** (1) : 13–39, 5 pls., Jan., 1911.) Reprint, 26 pp., 5 pls., June, 1916.
46. Crozier, W. J. — The Taste of Acids. Jour. Comp. Neurol., **26** (4) : 453–462. Aug., 1916.
47. Wenrich, D. H. — Notes on the Reactions of Bivalve Mollusks to Changes in Light Intensity : Image Formation in Pecten. Jour. Anim. Behavior, **6** (4) : 297–318. July–Aug., 1916.
48. Crozier, W. J. — Cell Penetration by Acids. II. Further Observations on the Blue Pigment of Chromodoris zebra. Jour. Biol. Chem., **26** (1) : 217–223. Sept., 1916.
49. Crozier, W. J. — Cell Penetration by Acids. III. Data on some Additional Acids. Jour. Biol. Chem., **26** (1) : 225–230. Sept., 1916.
50. Crozier, W. J. — On a Barnacle, Conchoderma virgatum, attached to a Fish, Diodon hystrix. Amer. Nat., **50** (598) : 636–639. Oct., 1916.
51. Crozier, W. J. — On the Immunity Coloration of some Nudibranchs. Proc. Nat. Acad. Sci., **2** (12) : 672–675. Dec., 1916.

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